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CLINICAL DIAGNOSIS OF RABBIT ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

In 2018, dead rabbits were continuously found in a rabbit farm in Anhui province; growing masses were observed in their oral cavity. This study aimed to show an oral diagnosis of oral squamous cell carcinoma in rabbits. Herein, diseased rabbits were dissected, the growing masses were histologically examined, and the papillomavirus was detected via PCR. These masses (multi-lobed, hard, and gray) were bilaterally present exclusively in the buccal area. Hematoxylin and eosin staining revealed obvious atypia in the cancer cells and infiltrative growth of the cancer tissue, yielding cancer cells with varying morphology (cancer nests). The large central region of the cancer nests displayed keratinization (keratin pearl). PCR analysis revealed negative findings for papillomavirus. Based on the results of dissection, histopathological analysis, and pathogen detection, these rabbits were finally diagnosed with squamous cell carcinoma. This study is the first report of oral squamous cell carcinoma in rabbits, thus laying the foundation for the diagnosis and studies on related diseases.

Key words: rabbit, oral squamous cell carcinoma, pathobiology.

INTRODUCTION

In recent years, with advancements in the diagnosis of veterinary diseases and food hygiene inspection, cancer-related diseases severely affecting animal health have received increasing attention among the medical, veterinary, and scientific personnel. With the development of the rabbit industry, the use of some rabbit species is relatively prolonged, and the risk of cancer-related diseases has relatively increased, similar to that in other animals. The incidence of cancer-related diseases in rabbits ranges from 0.5% to 2.7%, and common tumors in rabbits include uterine adenocarcinoma and breast and skin cancer (van Zeeland, 2017).

The most common oral malignant tumor is squamous cell carcinoma. As a common type of somatic tumor, squamous cell carcinoma not only causes facial deformity, but also has a high metastasis rate owing to abundant blood circulation and lymphatic reflux at the tumor site (Liping and Dong, 2018; Sun and Nan, 2019). The incidence of oral tumors is high in dogs and cats, accounting for 6% of all tumors (Yu *et al.*, 2008; Shen *et al.*, 2017; Li *et al.*, 2018). Rabbit oral tumors have recently been reported, most of which are benign oral tumors caused by rabbit oral papilloma virus (Sun and Yan, 2014), while rabbit malignant oral tumors including squamous cell carcinoma have not yet been reported (Chen, 1988; Wang and Chen, 1995; Chen *et al.*, 1998; van Zeeland, 2017). This study is the first to report oral squamous cell carcinoma in rabbits through an autopsy, pathological examination, and pathogen detection. This study is expected to further the current understanding of animal cancer-related diseases, especially rabbit oral cancer, and provide a foundation for the diagnosis and studies on related diseases.

MATERIALS AND METHODS

Material

Experimental Animals and reagents

Diseased rabbits were obtained from a fur rabbit farm in Anhui province. Trizol RNAiso plus, PrimeScript RT reagent Kit, and DNA Marker DL2000 were purchased from Takara (TaKaRa Bio, Kusatsu, Japan).

Methods

Disease Assessment

On visiting the Angora rabbit farm, farm owners and breeding personnel were interviewed to understand the incidence of the symptoms of rabbits including age, incidence, and other related conditions.

Autopsy and hematoxylin and eosin staining

Upon euthanasia, growing masses were surgically resected from the oral cavity of the rabbits and their morphology was observed. These masses were subjected to routine histopathological examination using 5-µm-thick sections and then stained with hematoxylin and eosin and digital images were obtained.

Pathogen examination

PCR primers were designed to amplify different DNA fragments of the papillomavirus. The reaction mixture was as follows: 2.5 µL 10×PCR buffer, 1 µL primer, 2 µL dNTP, 0.5 µL TaqDNA polymerase, and 5 µL of the extract, and the volume was adjusted to 25 µL with water. The cycling conditions were as follows: 94 °C for 5 min, followed by 32 cycles (94 °C for 30 s, 53 °C for 30 s, and 72 °C for 40 s), 72 °C for 5 min, and 4 °C for insulation.

RESULTS

Disease assessment

Rabbit characteristics

On interviewing the farm owners and staff, in the present farm and in surrounding farms, Angora rabbits (*Oryctolagus cuniculus*) were the predominant breed. No such disease occurred in meat rabbit, but rather in the breed of rabbits with long and curly fur. The age of the sick Angora rabbit was >2 years.

Clinical symptoms

Personal interviews of the farm owners and staff indicated that the tumors were large and resulted in facial deformities. The body temperature was normal; however, the oral tumors severely affected normal feeding. The sick rabbits were depressed, declined food, had difficulty in swallowing, salivating, and weight loss.

Autopsy

Autopsy revealed that the rabbit was fully developed and thin. The oral, nasal, and anal mucosae were pink, and the capillary filling time was normal. The oral tumors displayed infiltrative growth, resulting in facial swelling and deformation. The skin inside the oral cavity was mostly necrotic and bleeding was observed. The hyperplastic mass was hard on palpation, and was divided into multiple leaves. Upon careful examination, no growth was observed in the head or other parts of the body.

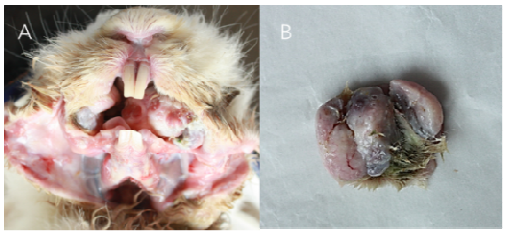

Figure 1: Growing masses in the oral cavity of rabbits.
A: masses growing bilaterally in the oral cavity. B: multiple leaves, pale section.

PCR pathogen detection

Total RNA was isolated from tumor tissue and reverse-transcribed into cDNA, which was used as the template. After PCR amplification with specific primers for papillomavirus, negative results were obtained (Figure 2). Combined with the clinical findings, it was preliminarily determined that the disease was not caused by a papillomavirus infection.

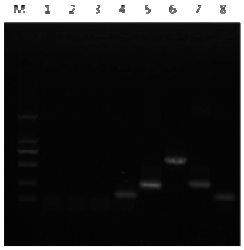

Figure 2: PCR amplification of different DNA fragments of papilloma virus CRPV
M: DNA maker (DL2000); 1–4: E3, E4, and E5 of papillary viral and rabbit cytochrome P450 were respectively amplified with tumor cDNA as a template; 5–8: E3, E4, and E5 and rabbit cytochrome P450 were amplified with papillomaviral DNA as a template.

Histopathological analysis

Hematoxylin and eosin staining revealed that the tumor tissues are invasive growth. Some of the nests are red with “keratin pearl” (yellow arrow) revealed the tissue of origin for this tumor, and the connective tissues are between the nests. The nests are composed of peripheral palisade columnar cells (black arrows) and central polygonal clear cells (red arrows). A small number of keratinized keratinocytes (yellow arrow) are also seen in the lesion (Figure 3). The organism was identified as squamous cell carcinoma.

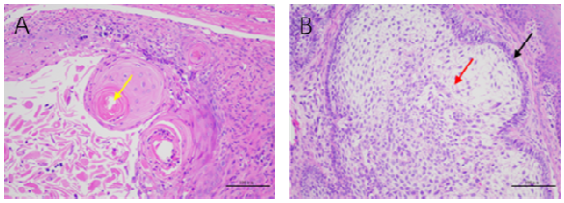

Figure 3 Squamous cell carcinoma in the oral cavity of rabbits.
A: the keratinized area of squamous cell carcinoma. B: non-keratinized squamous cell carcinoma lobules.

DISCUSSION

In the past decades, the rabbit industry has rapidly developed in China. With quality improvements in veterinary care and an increased rabbit lifespan, the number of rabbits diagnosed with tumors has also increased. Oral tumors have also been reported; however, their incidence seems to be low, mostly benign tumors resulting from viral infections (Peng and Hu, 2009; Liu, 2014; Sun and Yan, 2014).

This study furthers the current understanding of cancer-related diseases in animals, especially rabbit oral cancer, and provides a foundation for the diagnosis of and studies on related diseases in the future. However, the specific cause of this disease is unknown, being potentially associated with diet, microorganisms, genetic factors, and varieties (Li *et al*., 2015; D'Souza and Addepalli, 2018). The breeding time of Angora rabbits is usually more than 2 years. Because of the long breeding cycle, the probability of tumors is markedly increased. Addition, that breeding farmers preferably breed rabbits with curly fur, and this breed is more likely to develop malignant oral tumors; thus may be associated with genetic defects in these rabbits. Similar cases have been reported in studies in Yancheng city and other places, all of which involved Angora rabbits. Under the same conditions for fur growth, fur length was greater among susceptible rabbits than among normal rabbits, and the anterior end curled easily, thus further confirming that disease occurrence may have greater relevance to genetic factors.

Considering the economic factors and to prevent tumorigenesis and reduce the influence of cancer-related diseases in rabbits, the breeding personnel at the farms selectively bred dams not harboring tumors, while those harboring tumors were not bred and eliminated with time.

ACKNOWLEDGEMENTS

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