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REVIEW ARTICLE

Impact of Poxvirus Infection on Crocodile Skin Quality: A Systematic Review

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Abstract: Crocodile poxvirus infection causes small superficial lesions covered by dark brownish crusts or scabs. This infection is of importance in crocodile farming due to its potential impact on the tanned hide. The objective of this study was to systematically review literature on the effect of poxvirus infection on crocodile skin quality. The study systematically used four search engines: Google Scholar, PubMed, ScienceDirect, and Web of Science to search for literature. A total of five articles (n = 5) were included in the study. The results showed that only three (n = 3) articles reported that poxvirus infection has an effect on raw/tanned skin quality in crocodiles, depending on harvest time, as well as stage and severity of the infection. The limitation of this systematic review is that out of the five included articles, only three ascertained the impact of crocodile poxvirus on the raw/tanned skin. The systematic review concluded that poxvirus infection has an effect on the quality of crocodile hides.

Keywords: Crocodilian Viruses, Lesions, Exotic Leather, Systematic Review

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Introduction

Crocodile farming is a field of interest as it results in the production of valuable skins for international trade [1, 2]. However, crocodile skins are usually downgraded and sold at a lower economic value as a result of the presence of defects [2-4]. Some defects, such as the presence of “pit-holes”, are caused by poxvirus infection [2]. According to [1, 2] and, the presence of infectious diseases, such as poxvirus, poses a huge threat in crocodile farming as they may potentially affect the quality of the tanned hide. Research on poxvirus infections can help us understand how they impact the quality of skins, whether the lesions will eventually heal, and what management strategies can be implemented to mitigate the virus [2]. Gross and microscopic pathological findings in poxvirus-infected crocodiles can help us understand critical details about the virus’s life cycle and how they interact with host cells [1]. However, based on our knowledge, there is no systematic review reporting on the effect of poxvirus on the quality of crocodile skins. Hence, the objective of this study was to systematically review literature assessing the effect of poxvirus infection on crocodile skins. The study provides information that will help breeders and researchers in understanding the impact of poxvirus on the quality of crocodile skin, allowing for the implementation of precise disease management strategies to improve crocodile skin quality.

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Materials and Methods

Eligibility Criteria

"Crocodiles" were defined as the population of the study, with "poxvirus infection" as exposure and "skin quality" as outcomes. Prior to conducting the study, a preliminary search of Population, Exposure, and Outcome (PEO) components was conducted on Google Scholar.

Search Strategy

Two investigators conducted a scientific publication search in databases such as Google Scholar, PubMed, ScienceDirect, and Web of Science up to May 2024. The following combinations were used: "Poxvirus infection", "Crocodile poxvirus", "Poxvirus in crocodiles", or "Crocodilian viruses". Results were limited to the English language only.

Inclusion Criteria

The inclusion criteria included articles that assessed poxvirus lesions in crocodile species, articles written in English, and articles that focused on the keywords.

Exclusion Criteria

The exclusion criteria included articles that appeared as duplicates, articles that did not focus on crocodile species, articles without the keyword (poxvirus) in their title, and articles that had no available full text.

Data Extraction

The authors independently carried out data extraction of the current study and mutually agreed on all items. The included articles comprised an author, publication year, country, crocodile breed, and sample size.

Assessment of Risk of Bias

Joanna Briggs Institute (JBI) tool was used to assess the quality of the articles selected in this study. The assessment evaluated:

(1) Whether the sample inclusion criteria was well-defined

(2) Whether the experimental subjects and setting was thoroughly described

(3) Whether the exposure was measured accurately and reliably

(4) Whether standardized methods were used to assess the condition

(5) Whether confounding factors were identified

(6) Whether strategies to address these confounding factors were stated

(7) Whether the outcomes were measured reliably

(8) Whether a suitable statistical analysis was used

Each question was examined for each selected article and was answered with ‘Yes’ or ‘No’. The overall risk was classified as ‘Low’ or ‘Moderate’. The authors independently evaluated the risk and quality of each study.

Results

Figure 1 shows a summary of the identification and selection of articles at different phases of the review. Sixty-one (n = 61) articles were retrieved from several scientific search engines. From the sixty-one articles retrieved, twenty-one articles (n = 21) were removed due to duplication. The remaining thirty-five articles (n = 5) were screened for title, and twenty-five articles (n = 25) were removed. A total of ten articles (n = 10) were screened for abstract, and four articles (n = 4) were removed due to not focusing on either poxvirus infection, methods of examining the virus, area of lesion appearance, or the impact of the virus on the skin. The remaining six articles (n = 6) were screened for eligibility, with only one article (n = 1) removed due to lack of full text. As a result, only five articles (n = 5) were included.

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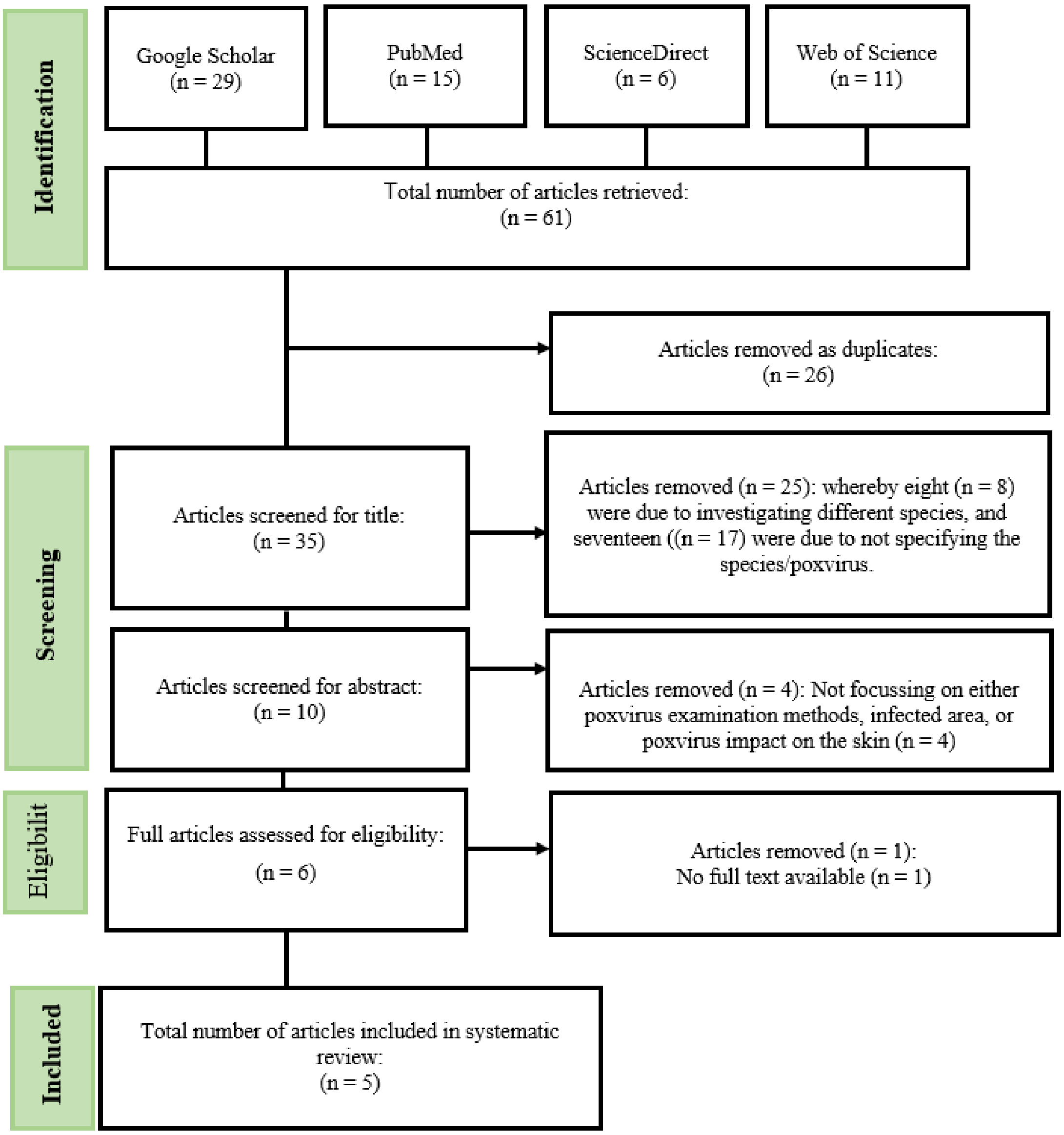

Fig. 1: Flow chart of identification and selection of studies used in the systematic review

Characterization of Included Studies

Table 1 shows the characteristics of the included articles of the systematic review. The findings indicated that out of the sixty-one (n = 61) articles, five (n = 5) articles were selected for inclusion in the systematic review. The included articles were published from 1990 to 2017, and one author appeared in more than one article [3, 4]. The articles included focused on varying sample sizes, ranging from 2 to 1500. Furthermore, the articles were done on crocodiles of different age groups, with at least 60% (3 out of 5 articles) of the articles focusing on 1-2 years old crocodile. Out of the five articles, only three articles (n = 3) reported the diameter of the poxvirus lesion, which ranged from 1- 6 mm, and 2mm appeared in at least two (n = 2) articles.

Origin of the Publications

Figure 2 shows the origin of the publications included in the systematic review. The findings indicated that Australia and South Africa were characterized by 40% (Australia = 2, South Africa = 2) of the studies each, while Zambia was characterized by the remaining 20% (n = 1).

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Table 1: Characterization of included studies

Author	Year	Country	Breed	N	Age	Lesion diameter	Area infected
[5]	1992	Australia	*Crocodylus porosus, Crocodylus johnstons*	2	12 months	1-2 mm	Hindfeet, belly and tail
[4]	1991	South Africa	*Crocodylus niloticus*	1500	7 months	3 mm	Body surface, tail, limbs, jaws and eyes
[3]	2009	South Africa	*Crocodylus niloticus*	4	Hatchling – 3 years	-	Smooth scales of the ventral skin
[2]	2017	Australia	*Crocodylus porosus*	14	<1 year ->2 years	-	Belly
[1]	1990	Zambia	*Crocodylus niloticus*	6	-	2-3 mm, up to 6 mm	Eyelids, mouth, nostrils, neck, belly, limbs and tail

Breed of Interest

Figure 3 shows the crocodile breed studied in the included articles. Out of the five articles included in the systematic review, 60% (n = 3) focused on *Crocodylus niloticus*, 40% (n = 2) focused on *Crocodylus porosus*, and 20% (n = 1) focused on *Crocodylus johnstoni*.

Area of the Target Lesions

Figure 4 shows the most common infected area on crocodiles as reported by the five articles included. Multiple areas of infection reported included hindfeet, belly, tail, jaws, eyes, limbs, nostrils, neck, teeth, and scales across the entire body surface. The belly, eyelids, limbs, and the tail were the most common areas of infection reported in three (60%) articles each. The mouth/jaws were the second most common area of infection, reported by two (40%) articles. The neck and nostrils were reported as infected areas in only one (20%) study each, making them the least common areas of infection.

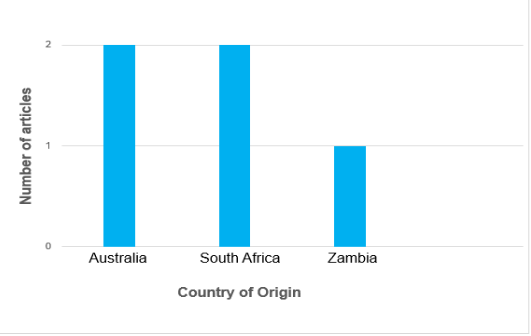

Fig. 2: Country of origin

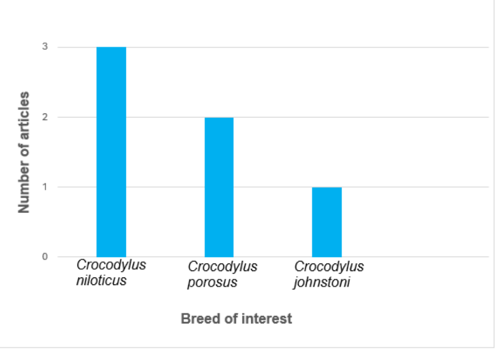

Fig. 3: Breed of interest

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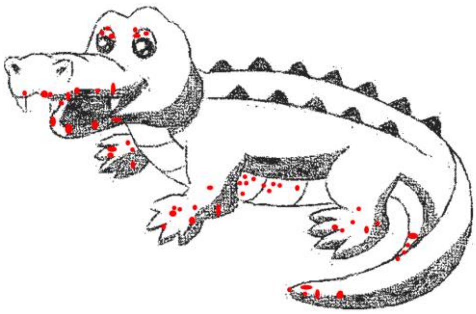

Fig. 4: Common areas of possible poxvirus lesions (Red dots represent the area in which the poxvirus lesions appeared

Microscopic Examination of Skin Lesion

Table 2 shows the microscopic examination used by the included articles for confirmation of poxvirus infection in crocodiles, using: Electron Microscope (EM) examination, Light Microscope (LM), Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Two articles (40%) used TEM, reporting dumb-bell shaped nucleoids in virions found in the epidermal cells [2, 5]. Furthermore, two articles (40%) employed electron microscopic (type not specified) examination and found that the cells comprised numerous virion particles [1, 4]. One study (20%) also subjected the dislodged lesions to SEM examinations, reporting largely packed virus particles in the inclusion bodies [3]. Furthermore, three articles (60%) also examined the poxvirus lesions using a light microscope [1, 3, 4].

Table 2: Microscopic examination of poxvirus lesions

Author and publication year	Type of microscope	Findings
[5]	TEM	Presence of elliptical virions, with nucleoids characterized by a dumb-bell shape.
[4]	LM and SEM	Inclusion bodies comprised of large virus particles densely packed
[3]	LM and EM	Lesions contain pox virions with cross-like patterns
[2]	TEM	Epidermal cells were enlarged and packed with mature virions, containing dumb-bell shaped nucleoids.
[1]	LM and EM	Cytoplasm of most prickle cells consisted of numerous poxvirus particles and matrices.

Macroscopic Examination of Crocodile Skin Lesions

Table 3 shows the findings resulting from the macroscopic examination of crocodile skins. Out of the five (n = 5) articles included in the review, two (40%) articles reported macroscopic findings following examination. Out of those two articles, one study reported the appearance of small deep pit holes on the ventral scales and sensory pores on the ventral scales situated close to its caudal edge [4]. The remaining study found that the skin had yellowish or brownish nodular lesions that appeared to be raised or unraised [1].

Table 3: Macroscopic examination of lesions on crocodile skin

Author and year of publication	Findings
[5]	N/A
[4]	N/A
[3]	Pin prick-like holes on smooth scales of the ventral skin and sensory pores close to its caudal edge
[2]	N/A
[1]	Unraised to raised plaque-like yellowish or brownish lesions

N/A means that the specified exam was not done

Histological Examination of Skin Lesions

Table 4 shows histological findings following examination of crocodile skins. Out of the five articles that met the inclusion criteria, only two (40%) articles reported histological findings. [2] reported intact keratin, disrupted keratin, depressed loci with abnormal keratin, and undulating dermal and epidermal layers in early active, active, expulsion, and healing phases, respectively. The other study found that histological findings showed hyperkeratosis and parakeratosis in epidermal lesions, as well as small neutrophilic granular and large eosinophilic homogeneous cytoplasmic inclusions in epithelial cells [1].

Histopathological Examination of Skin Nodules

Histopathological findings following examination of crocodile skins are shown in Table 5. Out of the five included articles, only three (60%) articles reported a histopathology examination. One study found that skin lesions were composed of thin and intact epidermis with eosinophilic cytoplasm [5]. The other study found that skin lesions comprised multiple large intracytoplasmic inclusions in the dermis with a mild dermal inflammatory reaction [3]. Skin lesions from hatchlings, 1-year-old, and 2-and-3-year-old crocodiles showed forming debris-filled cysts with intracytoplasmic inclusions in ballooning epidermal cells, collapsed cysts with a healed surface and infiltrations of lymphocytes under the epidermis, and collapsed cysts with complete epidermis that showed no sign of inflammatory reaction, respectively [4].

Examination of Skin Lesions using Molecular Characterization

Table 6 shows examination of crocodile skin lesions using molecular characterization. Out of the five articles chosen for inclusion, only two (40%) articles reported molecular characterization of the lesions. [4] reported that the purified and amplified Deoxyribonucleic Acid (DNA) sequence (533 bp), which aligned within the poxvirus ORF019 region, showed a 98.7% similarity and five differences at the amino acid (5 aa) level. The amplified sequences (159bp and 548bp) respectively aligned with Nile crocodile poxvirus regions, ORF19 and ORF99, resulting in 89% and 96% similarity [2].

Table 4: Histological examination of crocodile skin lesions

Author and year of publication	Findings
[5]	N/A
[4]	N/A
[3]	N/A
[2]	— Early active lesions showed epidermal hyperplasia and hypertrophy with overlying central plug (CP) of keratinocytes containing large eosinophilic “Henderson-Patterson-like” inclusion bodies. The overlying keratin was intact, and there was compression of the underlying loose dermis. — Active phase lesions showed further enlargement of CP with keratinocytes containing large “Henderson-Patterson-like” inclusions and disrupted keratin above CP, leaving an open hole. — Following expulsion phase, the lesions appeared grossly as large, depressed foci with abnormal keratin. — In the healing phase, the lesions were denoted by undulating dermal and epidermal layers as well as sparse chromatocytes.
[1]	— Large focal areas of marked acanthosis in epidermal lesions, accompanied by hyperkeratosis and parakeratosis. — Epithelial cells showed ballooning and had small neutrophilic granular and large eosinophilic homogeneous cytoplasmic inclusions.

N/A means that the specified exam was not done

Table 5: Histopathological examination of crocodile skin lesions

Author and year of publication	Findings
[5]	Skin lesions were composed of thin, intact epidermis overlying dermal accumulation of markedly enlarged cells with peripherally located nuclei, with intensely eosinophilic cytoplasm.
[4]	Skin lesions are comprised of multiple large intracytoplasmic inclusions in the dermis and a mild dermal inflammatory reaction.
[3]	— Two-and-three-year-old crocodiles: Skin lesions composed of collapsed cysts with complete epidermis that showed no sign of inflammatory reaction. — One-year-old crocodiles: Skin lesions consisted of a collapsed cyst with a healed surface; however, there were small perivascular infiltrations of lymphocytes under the epidermis. — Hatchlings: Skin showed the appearance of forming debris-filled cysts with intracytoplasmic inclusions in proliferating and ballooning epidermal cells.
[2]	N/A
[1]	N/A

N/A means that the specified exam was not done

Impact of Poxvirus Infection on Crocodile Skin

Table 7 shows the impact of poxvirus infection on the skin of crocodiles. Out of the five (5) included articles, only 60% (n = 3) ascertained the impact of poxvirus infection on crocodile skin quality, with one study reporting pits and scars on the tanned skin [4] and one reporting no effect on the skin but only if the lesions have healed prior to slaughtering [2]. The other study reported temporary scars and blemishes on the raw skin [3].

Table 6: Examination of crocodile skin lesions using molecular characterization

Author and year of publication	Base pairs (bp)	Poxvirus gene regions	Percentage similarity	Amino acid (aa) differences
[5]			N/A	
[4]			N/A	
[3]	533	ORF019	98.7%	5 aa
[2]	159	ORF19	89%	
	548	ORF99	96%	
[5]			N/A	

N/A means that the specified exam was not done

Table 7: Impact of poxvirus infection on crocodile skins

Author and year of publication	Findings
[5]	N/A
[4]	Temporary blemishes and scars, but no ascertained effect on the final skin product
[3]	Pit holes and scars
[2]	No effect upon healing
[1]	N/A

N/A means the article did not report on the topic

Discussion

Poxvirus infection is one of the most common crocodilian diseases and is of particular importance in crocodile farming due to its potential effect on quality of crocodile hides [2]. Hence, the objective of the study was to systematically review the literature on the effect of poxvirus infection in crocodiles. Understanding quality implications of the poxvirus on crocodile skin would assist in determining whether the lesions will indeed heal or determine the severity of biosecurity measures that may be required to control the infection [2, 4]. Five articles were included in this systematic review. The results showed that more articles were published in South Africa and Australia, and the most common breed of interest was Crocodylus niloticus (Nile crocodile). All the included articles (n = 5) reported that the poxvirus lesions appeared on the belly, tail, jaws, eyes, limbs, nostrils, neck, teeth, and scales across the entire body surface. The infection occurred mostly in yearlings. Notably, some species, including Saltwater, freshwater, and Nile crocodiles, may have been vulnerable to the poxvirus infection due to previously suffering from health conditions, such as fungal infiltrations, thermal granuloma, and salmonellosis [3, 5]. Out of the three (n = 3) articles that reported on the impact of poxvirus infection on crocodile skin, only one (n = 1) article ascertained the effect on the final tanned skin. On the other hand, one (n = 1) article showed that the skin would temporarily have scars and blemishes; however, the low inflammation of the epidermal wall could suggest that the tanned skin would not be poorly affected. Furthermore, one (n = 1) article showed that poxvirus infection will only affect the skin if the lesions did not heal. The results essentially show that harvest time plays a significant role, such that if infected crocodiles are slaughtered during the early stages of the virus, skin quality could potentially be poorly affected [2]. According to our knowledge, no systematic review has been done outlining the effect of poxvirus infection on the quality of crocodile skins. As a result, there are no comparisons of the current study with other systematic reviews. The result of the current study implies that factors such as harvest time and severity of poxvirus play a significant role in the overall impact of the virus on the skin of crocodiles [2, 4]. The strength of this study is that it is the first to systematically investigate the effect of poxvirus infection on the quality of crocodile skins. The limitation of this systematic review is that out of the five (n = 5) articles included, only three (n = 3) articles reported the effect of poxvirus infection on the raw/tanned crocodile skin. It can be concluded that poxvirus has an effect on the quality of crocodile skin and should, therefore, be taken into consideration when selecting crocodiles for slaughtering. Furthermore, strict biosecurity measures must be implemented to facilitate healing or mitigate the infection.

Conclusion

The study concluded that poxvirus infection influences the quality of crocodile skins. These findings will help farmers in the selection of crocodiles for slaughtering, thus minimizing economic losses of exotic skins at commercial markets. The study recommends:

(1) Further research using a larger sample size and other crocodile species

(2) Genetic testing of poxvirus lesions

(3) Comparing poxvirus with similar viral infections in crocodile species

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Author's Contributions

Pretty Rachuene and Thobela Louis Tyasi: Contributed to experimental design. Analysed the data and wrote the manuscript. Khetho Ratshilumela Nemutandani and Joseph Thinawanga Mugwabana: Revised and edited the manuscript. All the authors approved the final manuscript.

Ethics

All authors regarded plagiarism, misconduct, informed consent, data falsification, and fabrication as key ethical issues while conducting this systematic review.

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