Studies on Trueperella Pyogenes Isolated from an Okapi (Okapia johnstoni) and A Royal Python (Python Regius)

Marwa F.E. Ahmed
Mansoura University
Mazen Alssahen
Justus Liebig Universitat Giessen
Christoph Lämmler
Justus Liebig Universitat Giessen
Tobias Eisenberg
Landesbetrieb Hessisches Landeslabor
Madeleine Plötz
Tierarztliche Hochschule Hannover
Amir Abdulmawjood (amir.abdulmawjood@tiho-hannover.de)
Tierarztliche Hochschule Hannover https://orcid.org/0000-0001-5189-8381

Research article

Keywords: 16S rRNA gene, 16S-23S rDNA intergenic spacer region, LAMP, MALDI-TOF MS, pyolysin, rpoB, tuf

Posted Date: May 20th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-28967/v1

Version of Record: A version of this preprint was published at BMC Veterinary Research on August 14th, 2020. See the published version at https://doi.org/10.1186/s12917-020-02508-y.

Abstract

Background The present study was designed to characterize phenotypically and genotypically two *Trueperella pyogenes* strains isolated from an okapi (*Okapia johnstoni*) and a royal python (*Python regius*), respectively.

Results The species identity could be confirmed by phenotypic properties, by MALDI-TOF MS analysis and by detection of *T. pyogenes* chaperonin-encoding gene *cpn*60 with a previously developed loop-mediated isothermal amplification (LAMP) assay. Furthermore, sequencing of the 16S ribosomal RNA (rRNA) gene, the 16S-23S rDNA intergenic spacer region (ISR), the target genes *rpo*B encoding the β-subunit of bacterial RNA polymerase, *tuf* encoding elongation factor tu and *plo* encoding the putative virulence factor pyolysin allowed the identification of both *T. pyogenes* isolates at species level.

Conclusion Both strains could be clearly identified as *T. pyogenes*. The *T. pyogenes* strain isolated in high number from the vaginal discharge of an okapi seems to be of importance for the infectious process; the *T. pyogenes* strain from the royal python could be isolated from an apparently non-infectious process. However, both strains represent the first isolation of *T. pyogenes* from these animal species.

Background

Trueperella (*T.*) *pyogenes* is worldwide considered as part of the commensal biota of skin and mucous membranes of the upper respiratory and urogenital tract of animals (1). However, *T. pyogenes* is also an important opportunistic pathogen that causes mastitis, abortion and a variety of diverse pyogenic infections in livestock, including cattle, sheep, goats, horses, and pigs (2–4). In cattle, *T. pyogenes* appears to be responsible for infections of the reproductive tract (5) and the mammary gland (6), as well as cases of pneumonia and liver abscessation of bovines and small ruminants (7). In swine, *T. pyogenes* is well known as a causative agent of different types of inflammation in various organs including the lung, heart, joints, mammary glands, and in the reproductive tract (8, 9). Furthermore, *T. pyogenes* could be found in companion animals (4). One of the first reported cases in companion animals was an otitis externa detected in a cat and cystitis in a dog (10). More recently, Wareth et al (11) described a co-infection case of *T. pyogenes* with *Brucella abortus* in a cat and dog. Additionally, various wildlife animals could harbour *T. pyogenes* (3). In 2010, Ülbegi-Mohyla et al (12) characterized two *T. pyogenes* strains isolated from a bearded dragon and a gecko. Additionally, *T. pyogenes* infections were reported from a bison and from camels (13, 14), from goitered gazelles (15) and from a white-tailed deer (16). Likewise, some other sporadic cases of infectious diseases associated with *T. pyogenes* were described in a galago (17), in gray slender lorises (18, 19) and in a eurasian lynx (20).

Besides conventional bacteriological methods for identifiying *T. pyogenes* isolates, other new, fast and reliable techniques were described such as: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (20–23) Fourier transform infrared spectroscopy (FT-IR) (24), a loop-mediated isothermal amplification (LAMP) assay (25) and 16S rRNA gene sequencing (26, 27).

To date, only a small number of virulence factors in *T. pyogenes* have been recognized. Among these, *T. pyogenes* pyolysin encoded by gene *plo* appears to be one of the major virulence factors (28, 29).

Despite earlier studies being performed, further data on phenotypic and genotypic characteristics of *T. pyogenes* isolated from wildlife animals are needed. To the best of our knowledge, the present study provides a first detailed description of *T. pyogenes* recovered from an okapi and a royal python.

Results And Discussion

Both *T. pyogenes* strains investigated in the present study showed a narrow zone of complete hemolysis on 5% sheep blood agar and CAMP-like reactions in the staphylococcal β-hemolysin zone, with *Rhodococcus hoagii* as the indicator strain. The conventional biochemical properties and the results of the commercial identification system revealed almost identical results to previously investigated *T. pyogenes* of various origins and *T. pyogenes* DSM 20630^T (12, 18, 20, 30) (Table. 1). The *T. pyogenes* isolates gave positive reactions for pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, β-galactosidase, α-glucosidase and N-acetyl-β-glucosaminidase and negative reactions for nitrate reduction and pyrazinamidase. The isolates additionally hydrolyzed gelatine, but not esculin and urea. The isolates also fermented D-glucose, D-ribose, D-xylose, D-maltose, D-lactose and glycogen, but not D-mannitol. *T. pyogenes* 24398 fermented D-saccharose; however, *T. pyogenes* 171003246 was D-saccharose negative. In addition, both isolates showed a negative catalase reaction and a positive reaction on Löffler agar (Table. 1). A postitive reaction on Löffler agar is typical for *T. pyogenes* and widely used for phenotypic identification of this species (2, 18, 30, 31).

Table 1
Biochemical properties of *T. pyogenes* 24398 (okapi) and *T. pyogenes* 171003246 (royal python) investigated in the present study and type strain *T. pyogenes* DSM 20630^T.

Biochemical properties	***T. pyogenes* 24398 (okapi)**	***T. pyogenes* 171003246 (royal python)**	***T. pyogenes*[1] DSM 20630^T**
Haemolysis on SBA[2]	+	+	+
CAMP-like hemolytic reaction with[3]: *Staphylococcus aureus*	+	+	+
Streptococcus agalactiae	–	–	–
Rhodococcus hoagii	+	+	+
Reverse CAMP reaction	–	–	–
Nitrate reduction	–	–	–
Pyrazinamidase	–	–	–
Pyrrolidonyl Arylamidase	+	+	+
Alkaline phosphatase	+	+	+
β-Glucuronidase (β-GUR)	+	+	+
β-Galactosidase (β-GAL)	+	+	+
α-Glucosidase (α-GLU)	+	+	+
N-Acetyl-β-glucosaminidase (β-NAG)	+	+	+
Esculin (β-glucosidase)	–	–	–
Urease	–	–	–
Gelatine	+	+	+
Fermentation of:			
Glucose	+	+	+
Ribose	+	+	+

[1]Results taken from (Ülbegi-Mohyla et al. 2010; Hijazin et al. 2011; Eisenberg et al. 2012; Alssahen et al., 2020)

[2]SBA: Sheep Blood Agar

[3]synergistic or reverse CAMP-like reaction with indicator strains

Biochemical properties	*T. pyogenes* 24398 (okapi)	*T. pyogenes* 171003246 (royal python)	*T. pyogenes*[1] DSM 20630[T]
Xylose	+	+	+
Mannitol	–	–	–
Maltose	+	+	+
Lactose	+	+	+
Saccharose	–	+	–
Glycogen	+	+	+
Catalase	–	–	–
Serolysis on Loeffler agar	+	+	+
Identification % according to API-Coryne test System	99.9	99.9	99.9
[1]Results taken from (Ülbegi-Mohyla et al. 2010; Hijazin et al. 2011; Eisenberg et al. 2012; Alssahen et al., 2020)			
[2]SBA: Sheep Blood Agar			
[3]synergistic or reverse CAMP-like reaction with indicator strains			

+: positive reaction, –: negative reaction, [T]:type strain

Moreover, MALDI-TOF MS identified *T. pyogenes* 24398 and *T. pyogenes* 171003246 with log score values of 2.35 and 2.29 for the first hit and log score values of 2.28 and 1.9 for the second hit, respectively (data not shown). These log score values confirmed, in accordance with the current decision rules of the manufacturer, the species designation. Comparable to the present results, MALDI-TOF MS had already been shown to be a rapid and reliable technique for identifying bacteria of genera *Arcanobacterium* and *Trueperella*, including *T. pyogenes* (20, 21, 31).

The previously described *cpn*60-specific LAMP assay could successfully be used to identify the species-specific gene *cpn*60 of *T. pyogenes* 24398 and *T. pyogenes* 171003246 in the present investigation. This was comparable to the LAMP assay for detecting gene *cpn*60 of the previously described *T. pyogenes* of various origins (25), a *T. pyogenes* strain isolated from an adult roebuck (*Capreolus capreolus*) (23), and a *T. pyogenes* strain isolated from a eurasian lynx (*Lynx lynx*) (20). The results of the *cpn*60 LAMP assay are shown in Fig. 1 and Table. 2.

The oligonucleotide primers, 16SUNI-L and 16SUNI-R, were used for amplifying of 16S rRNA gene of the investigated *T. pyogenes* isolates. The nucleotide sequence data of *T. pyogenes* 24398 (GenBank accession numbers: MN946520) and *T. pyogenes* 171003246 (MN712476) were compared with type strain *T. pyogenes* DSM 20630^T (AAC45754) and with the previously described strain *T. pyogenes* S 1276/1/18 isolated from a eurasian lynx (MN135984), *T. abortisuis* DSM 19515^T (FN667628), *T. bernardiae* DSM 9152^T (X79224), *T. bialowiezensis* DSM 17162^T (EU194569), and *T. bonasi* DSM 17163^T (EU194570). The nucleotide sequence data of *T. pyogenes* 24398 and *T. pyogenes* 171003246 revealed a sequence homology of 98.9% among both strains, a sequence homology of 99.5% and 98.7% with *T. pyogenes* DSM 20630^T, and a sequence homology of 99.9% and 99.1% with *T. pyogenes* S1276/1/18, respectively. The control strains of genus *Trueperella* yielded a sequence homology to both *T. pyogenes* isolates ≤ 98.7% (Fig. 2).

Table 2
Results of LAMP including detection time and annealing temperature of the tested isolate, positive and negative control.

Sample ID	*T. pyogenes* 24398	*T. pyogenes* 171003246	*T. pyogenes* DSM 20630^T	*T. abortisuis* DSM 19515^T	*T. bernardiae* DSM 9152^T	*T. bonasi* DSM 17163^T	HPLC water and Master mix
Result	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Detection time hh:mm:ss	00:11:00	00:08:30	00:10:00	0	0	0	0
Annealing	89.6	89.4	89.9	0	0	0	0

+ve: Positive, -ve: Negative, ^{T}:type strain

Both strains *T. pyogenes* 24398 and *T. pyogenes* 171003246 were further identified by sequencing ISR, the genes *tuf* and *rpo*B and the putative virulence factor pyolysin encoding gene *plo*. *T. pyogenes* 24398 and *T. pyogenes* 171003246 showed sequence similarities of ISR (MN947249, MN724920) of 99.8% and 98.9% with *T. pyogenes* DSM 20630^T (EU194563) and 100% and 99.8% with *T. pyogenes* S 1276/1/18 (MN164031), respectively with 98.5% identity between both strains. The additionally investigated gene *tuf* (MN956808, MN741111) showed a sequence similarity of 99.6% and 99.7% with *T. pyogenes* DSM 20630^T (HG941716), and 99.6% and 99.7% with *T. pyogenes* S 1276/1/18 (MN163266), respectively; gene *rpo*B (MN956807, MN741109), a sequence similarity of 99.8% and 98.3% with *T. pyogenes* DSM 20630^T (FN550375), and 98.8% and 98.3% with *T. pyogenes* S 1276/1/18 (MN163265), respectively, and gene *plo* (MN956808, MN741110), a sequence similarity of 99.5% with *T. pyogenes* DSM 20630^T (U84782) for both isolates, and 99.1% with *T. pyogenes* S 1276/1/18 (MN163264) for both isolates. A dendrogram analysis of both ISR and the genes *tuf* and *rpo*B is presented in Fig. 3a, b c.

A phylogenetic analysis of the amino acid sequences of pyolysin (PLO) encoded by gene *plo* of *T. pyogenes* 24398 (MN956806), and *T. pyogenes* 171003246 (MN741110) investigated in the present study with PLO of type strain *T. pyogenes* DSM 20630^T (AAC45754), PLO of *T. pyogenes* S 1276/1/18 (MN163264), arcanolysin (ALN) of *Arcanobacterium haemolyticum* (ACV96715), phocaelysin (PHL) of *Arcanobacterium phocae* 10002^T (SMR98720), listeriolysin O (HLY) of *Listeria monocytogenes* (NP_463733), intermedilysin (ILY) of *Streptococcus intermedius* (BAA89790), pneumolysin (PLY) of *Streptococcus pneumoniae* (ADF28298) and streptolysin O (SLO) of *Streptococcus pyogenes* (BAB41212) obtained from the NCBI GenBank showed an amino acid similarity of *T. pyogenes* 24398 and *T. pyogenes* 171003246 of 99.5% with PLO of *T. pyogenes* DSM 20630^T and 99.1% with PLO of *T. pyogenes* S 1276/1/18 and was less pronounced to sequences of the other pore-forming toxins (Fig. 4).

Conclusion

T. pyogenes 24398 was isolated in high numbers from vaginal discharge of an okapi and seems to be responsible for the infectious process; *T. pyogenes* 171003246 was isolated from a non-infectious process of a royal python suffering from a throat swelling, possibly caused by traumatic reasons. Both *T. pyogenes* could be correctly identified by a phenotypical test, by MALDI-TOF MS analysis and by investigating the genomic targets, 16S rRNA gene, ISR, *tuf*, *rpo*B and plo giving a first detailed characterization of single *T. pyogenes* strains of this origin.

Methods

Bacterial strains

T. pyogenes 24398 was isolated in 2019 in high numbers (+++), together with *Enterobacter cloacae* (+) and *Pasteurella* sp. (+) from a living okapi (*Okapia johnstoni*) at Frankfurt Zoo, Frankfurt am Main, Germany. The sample was obtained during routine diagnostics of the animal. *T. pyogenes* 171003246 was recovered in 2017 in low numbers (+) by post-mortem analysis from the kidney of a dead seven-year-old female royal python (*Python regius*) measuring the length of 107 cm and a weighing 1.23 kg from a bird park in Hesse, Germany, together with *E. coli* (+), α-hemolytic streptococci (+), *Corynebacterium* sp. (+) and *Clostridium sardiniense* (+). The post-mortem analysis of the royal python revealed a good body condition and in the throat and head area a 15 cm lung edema and swelling, possibly caused by traumatic reasons. The post-mortem analysis of royal python, bacterial cultivation and preliminary identification of both bacteria were performed at Landesbetrieb Hessisches Landeslabor (LHL) Gießen, Germany. Both *T. pyogenes* strains were further investigated phenotypically and genotypically.

Phenotypic properties

A phenotypic characterization was performed using conventional cultural and biochemical assays as previously described (12, 18, 20, 30) and the API-Coryne test System (BioMérieux, Nürtingen, Germany) in accordance with the manufacturer's instructions. Furthermore, the bacterial isolates were identified by

MALDI-TOF MS using a Microflex LT (Bruker Daltonik GmbH, Bremen, Germany) instrument and MBT Compass Explorer 4.1 software (Bruker Daltonik GmbH). Sample preparation was carried out in accordance with the manufacturer's instructions using the direct transfer method. Briefly, one microbial colony was first smeared in duplicate onto spots of the MALDI target MSP 96 target (MicroScout Target plate; Bruker Daltonik GmbH) with sterile toothpicks. The air-dried bacteria were overlaid with 1 µL of an α-cyan 4-hydroxycinnamic acid matrix solution (HCCA, in 50% acetonitrile and 2.5% trifluoroacetic acid in pure water) followed by drying and loading into the mass spectrometer.

Genotypic properties

DNA extraction

The genomic DNA of both isolates and the type strains *T. pyogenes* DSM 20630^T, *T. abortisuis* DSM 19515^T, *T. bernardiae* DSM 9152^T and *T. bonasi* DSM 17163^T were extracted using the DNeasy blood and tissue kit (Qiagen GmbH, Hilden, Germany), in accordance with the manufacturer's instructions. The concentration and purity of DNA were measured by means of a Nano Drop spectrophotometer (ND1000; Thermo Fisher Scientific GmbH, Dreieich, Germany).

Determination of T. pyogenes chaperonin 60 encoding gene (cpn60)

The detection of gene *cpn*60 of *T. pyogenes* was performed using a previously designed loop-mediated isothermal amplification (LAMP) assay (25) with a portable real-time fluorometer (Genie II®, OptiGene Ltd, Horsham, UK) and the reference strains *T. pyogenes* DSM 20630^T, *T. abortisuis* DSM 19515^T, *T. bernardiae* DSM 9152^T and *T. bonasi* DSM 17163^T.

Further genotypic properties

Both *T. pyogenes* isolates were also evaluated by PCR for the presence of five genomic targets: 16S rRNA gene (16S), 16S-23S rDNA intergenic spacer region (ISR), the β-subunit of bacterial RNA polymerase encoding gene *rpo*B, the elongation factor tu encoding gene *tuf*, and pyolysin encoding gene *plo*. The sequence of the oligonucleotide primers and the PCR condition were previously described by Hassan et al. (26), Ülbegi-Mohyla et al. (12), Hijazin et al. (30), Eisenberg et al. (18), Wickhorst et al. (31), Wickhorst et al. (23), Alssahen et al. (20).

The PCR products were purified and sequenced by Eurofins Umwelt Nord GmbH (Göttingen, Germany). The obtained sequences of the different genes of the *T. pyogenes* isolates were aligned and further analyzed using the cluster method of the MegAlign program (DNASTAR Inc., ver. 15, Madison, WI, USA) and compared with the nucleotide sequences of the targets 16S rRNA, ISR, *rpo*B, *tuf* and *plo* of different *Trueperella* reference strains obtained from the NCBI GenBank. Moreover, the resulting amino acid sequences of pyolysin of both *T. pyogenes* isolates were compared with the amino acid sequences of pyolysin of *T. pyogenes* DSM 20630^T, closely related pore-forming toxins of genus *Arcanobacterium* and with other bacterial pore-forming toxins obtained from the NCBI GenBank.

Declarations

Ethics approval and consent to participate

This study did not require official or institutional ethical approval. The material was collected post mortem and / or during routine diagnosis. According to competent authorities, this kind of research does not require ethics approval or general approval with respect to German law.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

M.F.E.A, M.A., C.L., A.A. and M.P. contributed to the design of the study, collected and analysed the data. T.E. performed the initial examination of the isolates. M.F.E.A, A.A. and M.P. drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Authors' information (optional)

[1]Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

[2]Institut für Hygiene und Infektionskrankheiten der Tiere, Justus-Liebig-Universität Gießen, Frankfurterstraße 85-91, D-35392 Gießen, Germany

[3]Landesbetrieb Hessisches Landeslabor (LHL), Schubertstraße 60, D-35392 Gießen, Germany

[4]Institute of Food Quality and Food Safety, Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany

References

1. Rzewuska M, Kwiecien E, Chrobak-Chmiel D, Kizerwetter-Swida M, Stefanska I, Gierynska M. Pathogenicity and Virulence of *Trueperella pyogenes*: A Review. Int J Mol Sci. 2019; 20(11).
2. Lämmler C, Hartwigk H. Actinomyces pyogenes und *Arcanobacterium haemolyticum*. In: Handbuch der bakteriellen Infektionen bei Tieren. 2 edn edn.: Gustav Fischer Jena 1995: 196–240.
3. Jost BH, Billington SJ. *Arcanobacterium pyogenes*: molecular pathogenesis of an animal opportunist. Antonie Van Leeuwenhoek. 2005;88(2):87–102.
4. Ribeiro MG, Risseti RM, Bolanos CAD, Caffaro KA, de Morais ACB, Lara GHB, et al. *Trueperella pyogenes* multispecies infections in domestic animals: a retrospective study of 144 cases (2002 to 2012). Vet Quart. 2015;35(2):82–7.
5. Tamai IA, Mohammadzadeh A, Salehi TZ, Mahmoodi P. Genomic characterisation, detection of genes encoding virulence factors and evaluation of antibiotic resistance of *Trueperella pyogenes* isolated from cattle with clinical metritis. Antonie Van Leeuwenhoek. 2018;111(12):2441–53.
6. Ishiyama D, Mizomoto T, Ueda C, Takagi N, Shimizu N, Matsuura Y, et al. Factors affecting the incidence and outcome of *Trueperella pyogenes* mastitis in cows. J Vet Med Sci. 2017;79(3):626–31.
7. Rogovskyy AS, Lawhon S, Kuczmanski K, Gillis DC, Wu J, Hurley H, et al. Phenotypic and genotypic characteristics of *Trueperella pyogenes* isolated from ruminants. J Vet Diagn Invest. 2018;30(3):348–53.
8. Jarosz LS, Gradzki Z, Kalinowski M. *Trueperella pyogenes* infections in swine: clinical course and pathology. Pol J Vet Sci. 2014;17(2):395–404.
9. Dong WL, Liu L, Odah KA, Atiah LA, Gao YH, Kong LC, et al. Antimicrobial resistance and presence of virulence factor genes in *Trueperella pyogenes* isolated from pig lungs with pneumonia. Trop Anim Health Pro. 2019;51(7):2099–103.
10. Billington SJ, Post KW, Jost BH. Isolation of *Arcanobacterium* (*Actinomyces*) *pyogenes* from cases of feline otitis externa and canine cystitis. J Vet Diagn Invest. 2002;14(2):159–62.
11. Wareth G, El-Diasty M, Melzer F, Murugaiyan J, Abdulmawjood A, Sprague LD, et al. *Trueperella pyogenes* and *Brucella abortus* Coinfection in a Dog and a Cat on a Dairy Farm in Egypt with Recurrent Cases of Mastitis and Abortion. Vet Med Int. 2018;2018:2056436.
12. Ülbegi-Mohyla H, Hijazin M, Alber J, Lämmler C, Hassan AA, Abdulmawjood A, et al. Identification of *Arcanobacterium pyogenes* isolated by post mortem examinations of a bearded dragon and a gecko by phenotypic and genotypic properties. J Vet Sci. 2010;11(3):265–7.
13. Wareth G, Murugaiyan J, Khater DF, Moustafa SA. Subclinical pulmonary pathogenic infection in camels slaughtered in Cairo, Egypt. J Infect Dev Countr. 2014;8(7):909–13.
14. Tarazi YH, Al-Ani FK. An outbreak of dermatophilosis and caseous lymphadenitis mixed infection in camels (*Camelus dromedaries*) in Jordan. J Infect Dev Countr. 2016;10(5):506–11.
15. Òªeò«en G, Buyukcangaz EK, Caliskan UG, Cangul TI, Akdesir E. Interdigital Necrobacillosis Associated with *Trueperella Pyogenes* in Goitered Gazelles (*Gazella Subgutturosa*). J Zoo Wildlife

Med. 2018;49(2):429–34.

16. Cohen BS, Belser EH, Keeler SP, Yabsley MJ, Miller KV. Isolation and Genotypic Characterization of *Trueperella* (*Arcanobacterium*) *Pyogenes* Recovered from Active Cranial Abscess Infections of Male White-Tailed Deer (*Odocoileus Virginianus*). J Zoo Wildlife Med. 2015;46(1):62–7.
17. Salleng KJ, Burton BJ, Apple TM, Sanchez S. Isolation of *Trueperella pyogenes* in a case of thoracic and abdominal abscess in a galago (*Otolemur garnettii*). J Med Primatol. 2016;45(4):198–201.
18. Eisenberg T, Nagib S, Hijazin M, Alber J, Lämmler C, Hassan AA, et al. *Trueperella pyogenes* as cause of a facial abscess in a grey slender loris (*Loris lydekkerianus nordicus*) - a case report. Berl Munch Tierarztl. 2012;125(9–10):407–10.
19. Nagib S, Glaeser SP, Eisenberg T, Sammra O, Lämmler C, Kampfer P, et al. Fatal infection in three Grey Slender Lorises (*Loris lydekkerianus nordicus*) caused by clonally related *Trueperella pyogenes*. BMC Vet Res. 2017;13–273.
20. Alssahen M, Peters M, Rau J, Hassan AA, Sammra O, Lämmler C, et al. Phenotypic and genotypic approach to characterize a *Trueperella pyogenes* strain isolated from an Eurasian Lynx (*Lynx lynx*). Berl Munch Tierarztl. 2020, In press.
21. Hijazin M, Hassan AA, Alber J, Lämmler C, Timke M, Kostrzewa M, et al. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for species identification of bacteria of genera *Arcanobacterium* and *Trueperella*. Vet Microbiol. 2012;157(1–2):243–5.
22. Randall LP, Lemma F, Koylass M, Rogers J, Ayling RD, Worth D, et al. Evaluation of MALDI-TOF as a method for the identification of bacteria in the veterinary diagnostic laboratory. Res Vet Sci. 2015;101:42–9.
23. Wickhorst JP, Hassan AA, Sheet OH, Eisenberg T, Sammra O, Alssahen M, et al. *Trueperella pyogenes* isolated from a brain abscess of an adult roebuck (*Capreolus capreolus*). Folia Microbiol. 2018;63(1):17–22.
24. Nagib S, Rau J, Sammra O, Lämmler C, Schlez K, Zschock M, et al. Identification of *Trueperella pyogenes* Isolated from Bovine Mastitis by Fourier Transform Infrared Spectroscopy. Plos One. 2014;9(8).
25. Abdulmawjood A, Wickhorst J, Hashim O, Sammra O, Hassan AA, Alssahen M, et al. Application of a loop-mediated isothermal amplification (LAMP) assay for molecular identification of *Trueperella pyogenes* isolated from various origins. Mol Cell Probe. 2016;30(4):205–10.
26. Hassan AA, Ülbegi-Mohyla H, Kanbar T, Alber J, Lämmler C, Abdulmawjood A, et al. Phenotypic and genotypic characterization of *Arcanobacterium haemolyticum* isolates from infections of horses. J Clin Microbiol. 2009;47(1):124–8.
27. Moreno LZ, Matajira CEC, da Costa BLP, Ferreira TSP, Silva GFR, Dutra MC, et al. Characterization of porcine *Trueperella pyogenes* by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), molecular typing and antimicrobial susceptibility profiling in Sao Paulo State. Comp Immunol Microb. 2017;51:49–53.

28. Billington SJ, Songer JG, Jost BH. Molecular characterization of the pore-forming toxin, pyolysin, a major virulence determinant of *Arcanobacterium pyogenes*. Vet Microbiol. 2001;82(3):261–74.
29. Ding H, Lämmler C. Purification and further characterization of a haemolysin of *Actinomyces pyogenes*. J Vet Med B. 1996;43(3):179–88.
30. Hijazin M, Ülbegi-Mohyla H, Alber J, Lämmler C, Hassan AA, Abdulmawjood A, et al. Molecular identification and further characterization of *Arcanobacterium pyogenes* isolated from bovine mastitis and from various other origins. J Dairy Sci. 2011;94(4):1813–9.
31. Wickhorst JP, Sammra O, Hassan AA, Alssashen M, Lämmler C, Prenger-Berninghoff E, et al. Identification of *Arcanobacterium hippocoleae* by MALDI-TOF MS analysis and by various genotypical properties. Res Vet Sci. 2017;115:10–2.

Figures

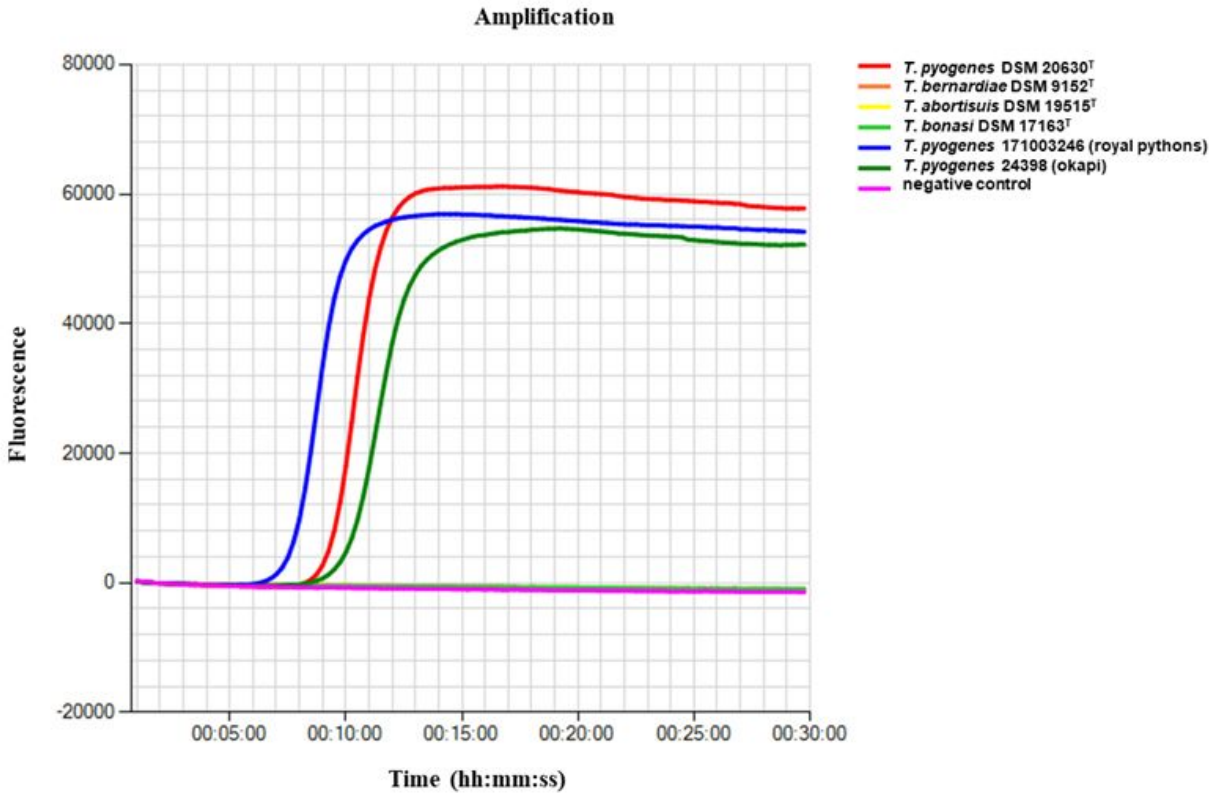

Figure 1

Positive LAMP assay of T. pyogenes 24398 (okapi), T. pyogenes 171003246 (royal python), T. pyogenes DSM 20630T, the LAMP negative control strains T. abortisuis DSM 19515T, T. bernardiae DSM 9152T and T. bonasi DSM 17163T, and a negative control.

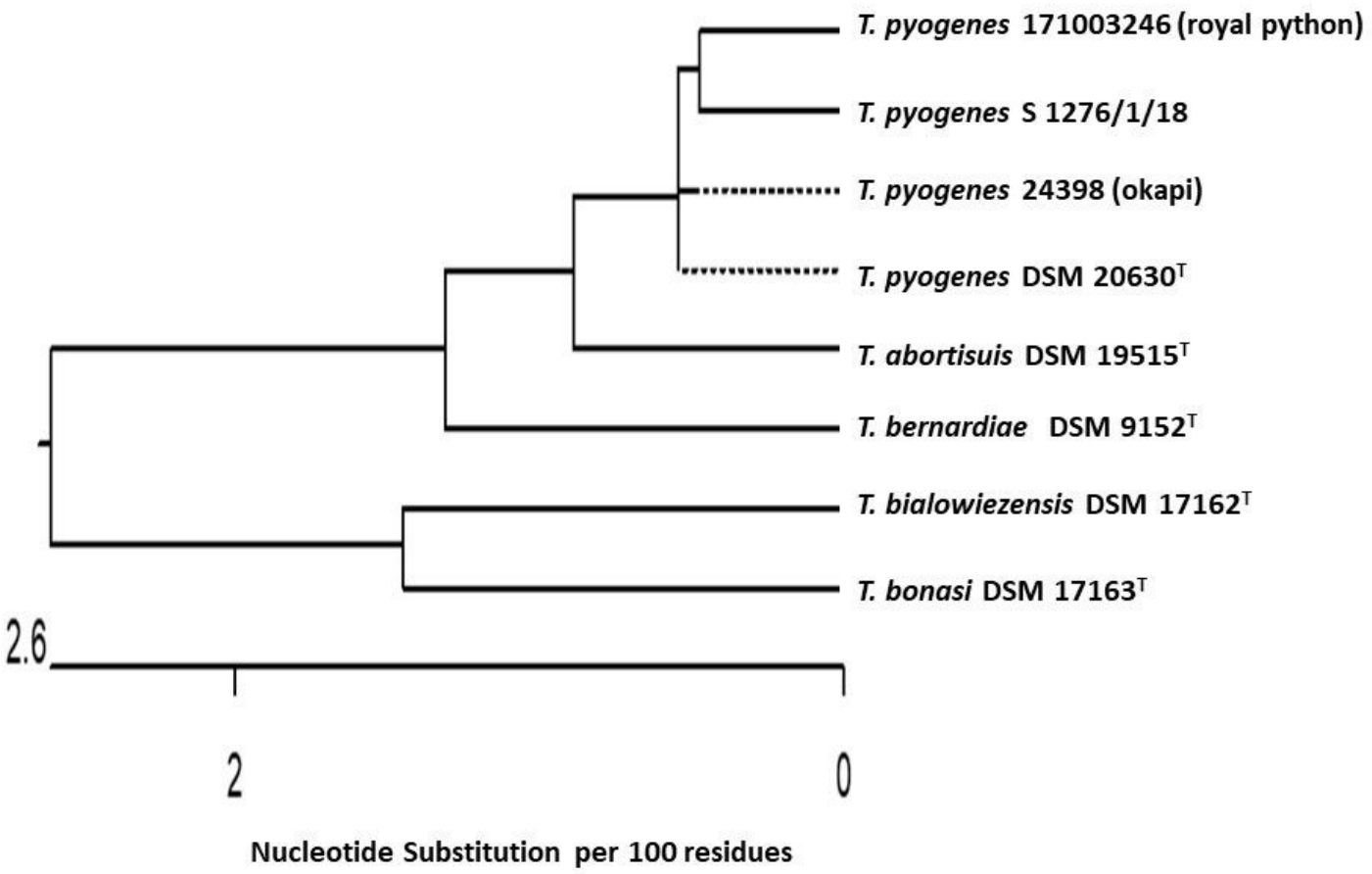

Figure 2

Phylogenetic analysis based on nucleotide sequences of 16S rRNA gene of the investigated T. pyogenes 24398 and T. pyogenes 171003246 isolated from okapi and royal python compared with the type strain T. pyogenes DSM 20630T and T. pyogenes S 1276/1/18 isolated from a eurasian lynx, T. abortisuis DSM 19515T, T. bernardiae DSM 9152T, T. bialowiezensis DSM 17162T, and T. bonasi DSM 17163T.

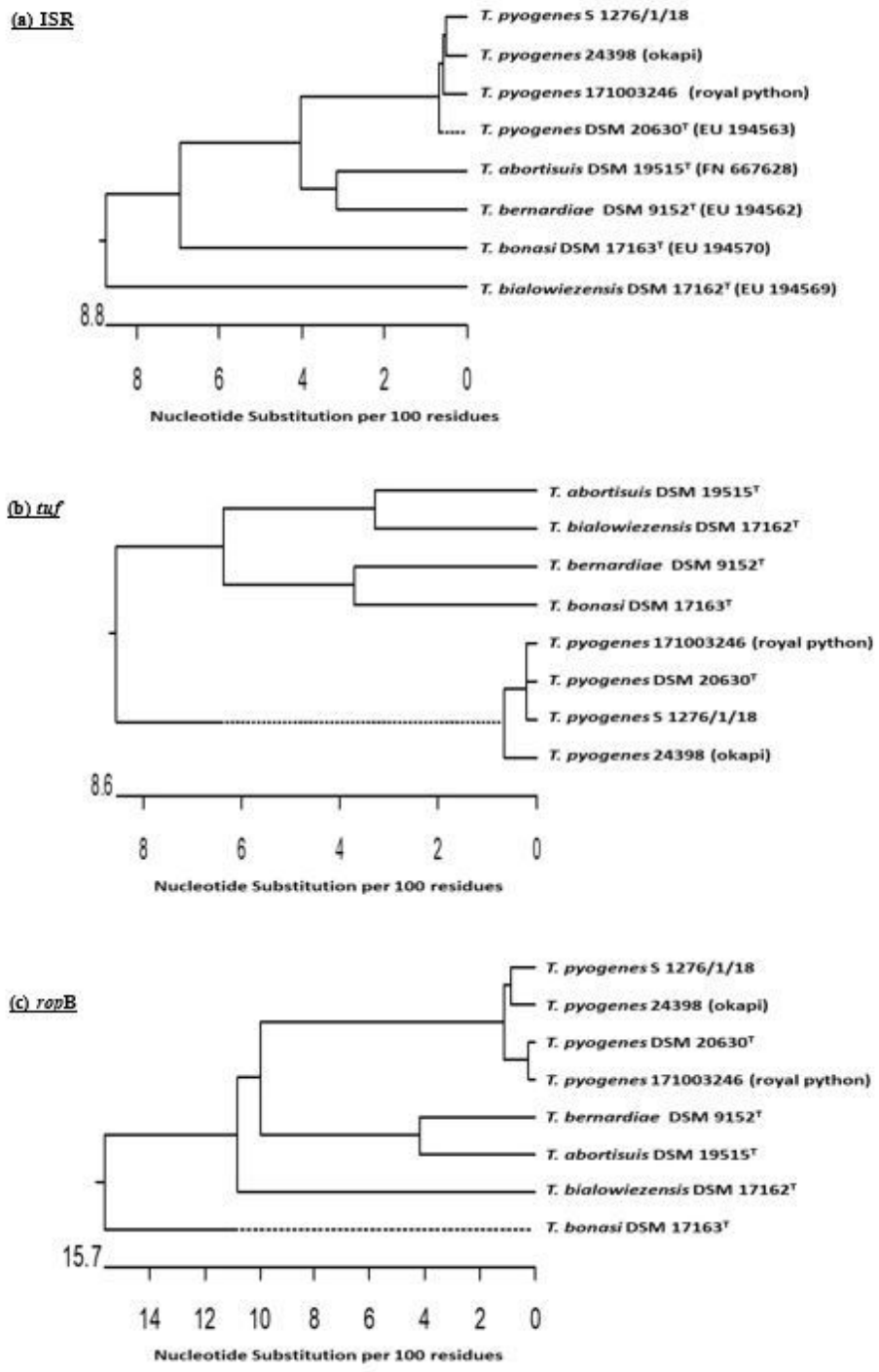

Figure 3

Phylogenetic analyses based on ISR (a), tuf (b) and ropB (c) nucleotide sequences of the investigated T. pyogenes 24398 and T. pyogenes 171003246 and T. pyogenes S 1276/1/18 isolated from a eurasian lynx and the control strains, T. pyogenes DSM 20630T, T. abortisuis DSM 19515T, T. bernardiae DSM 9152T, T. bialowiezensis DSM 17162T, and T. bonasi DSM 17163T.

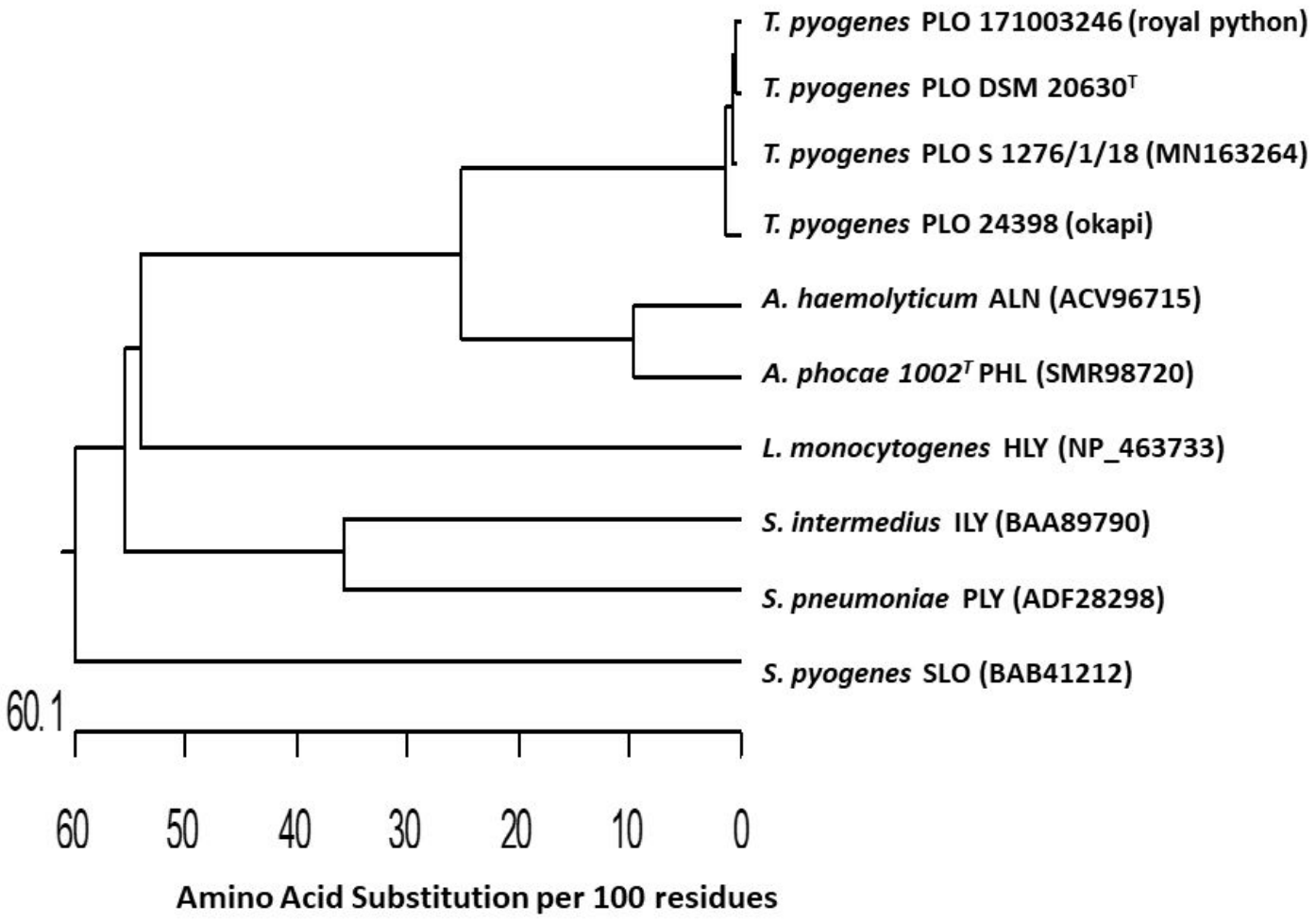

Figure 4

Phylogenetic relationships among amino acid sequences PLO of the investigated T. pyogenes 24398 and T. pyogenes 171003246, PLO of type strain T. pyogenes 20630T, T. pyogenes S 1276/1/18 isolated from a eurasian lynx, ALN of A. haemolyticum, PHL of A. phocae, HLY of L. monocytogenes, PLY of S. pneumoniae, ILY of S. intermedius, and SLO of S. pyogenes obtained from the NCBI GenBank.