2012

Rhodotorula minuta fungemia in a ewe lamb

C G. Chitko-McKown
USDA-ARS, carol.chitkomckown@ars.usda.gov

K A. Leymaster
USDA-ARS

M. P. Heaton
USDA-ARS, mike.heaton@usda.gov

D D. Griffin
University of Nebraska-Lincoln

J K. Veatch
Murray State University

See next page for additional authors

Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

SHORT COMMUNICATION

Rhodotorula minuta fungemia in a ewe lamb

C. G. Chitko-McKown[1], K. A. Leymaster[1], M. P. Heaton[1], D. D. Griffin[2], J. K. Veatch[3], S. A. Jones[1] and M. L. Clawson[1]

[1] USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA
[2] Great Plains Veterinary Education Center, University of Nebraska, Lincoln, NE, USA
[3] Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, USA

Keywords:
fungemia; sheep; *Rhodotorula minuta*

Correspondence:
Carol G. Chitko-McKown. USDA-ARS, U.S. Meat Animal Research Center, PO Box 166, Clay Center, NE 68933, USA.
Tel.: +1 402 762 4372;
Fax: +1 402 762 4390;
E-mail: carol.chitkomckown@ars.usda.gov

Received for publication October 29, 2012

doi:10.1111/tbed.12062

Summary

An 8-month-old crossbred ewe, normal upon physical examination, was humanely euthanized for tissue collection. After approximately 3 weeks in tissue culture, fungi began budding out of cells obtained from the choroid plexus. After an additional 3 weeks, budding was observed in kidney cell cultures and eventually in monocyte cultures as well. Serum from the lamb was submitted to the Veterinary Diagnostic Laboratory at Colorado State University for fungal diagnosis and was found negative for *Aspergillus*, *Blastomyces*, *Coccidioidomycosis* and *Histoplasmosis*. DNA was isolated from fungi collected from tissue culture supernatants and used in a set of pan-fungal PCR assays with DNA from *Candida* acting as a positive control. PCR products were sequenced and BLAST analysis performed. The unknown fungal sequence aligned with 100% identity to *Rhodotorula minuta* an emerging opportunistic pathogen. Samples were submitted to The Fungal Testing Laboratory at The University of Texas Health Science Center at San Antonio for additional validation. We believe this to be the first report of *Rhodotorula* fungemia in a sheep in the United States.

The Study

Experimental procedures were approved and performed in accordance with U.S. Meat Animal Research Center Animal Care Guidelines and the Guide for Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010). An 8-month-old, crossbred ewe lamb that was normal upon physical examination was humanely euthanized for tissue collection to develop cell lines for an ongoing Ovine Progressive Pneumonia study. Before euthanasia via intravenous infusion of Beuthanasia-D Special (Intervet Inc., Merck Animal Health, Summit, NJ, USA), whole blood was collected via jugular venipuncture into 60-ml syringes containing EDTA anticoagulant. After euthanasia, the brain was removed and choroid plexus harvested. The internal organs appeared normal upon gross examination, and 1 kidney was removed. The choroid plexus and kidney were immediately placed into sterile 50-ml tubes containing ice-cold phosphate-buffered saline and were transported to the laboratory for cell isolation. Monocytes were purified from whole blood by density gradient centrifugation (Chitko-McKown et al., 2004), and cell suspensions were obtained from the choroid plexus and kidney by mincing the tissue and digestion in 0.25% trypsin (Chitko-McKown and Macneil, 2010). Each tissue-specific cell suspension was placed in a 75 cm^2 tissue culture flask in RPMI culture medium with antibiotic and fungizone, L-glutamine and either 10% (choroid plexus and kidney fibroblasts) or 5% (monocytes) foetal bovine serum and was cultured at 37°C, 7% CO_2 in a humidified atmosphere. After approximately 3 weeks of culture, fungi began budding from cells obtained from the choroid plexus (Fig. 1). After an additional 3 weeks, the budding was observed in the kidney cell cultures and eventually in the monocyte cultures as well (Fig. 2). Fungus was isolated from the cell cultures by collecting the supernatant from infected cultures and pelleting the fungus by centrifugation at 1200 ***g*** for 15 min at 4°C. Cell monolayers of all lineages appeared healthy, and after fungi removal and medium replacement, continued to grow. Because we had

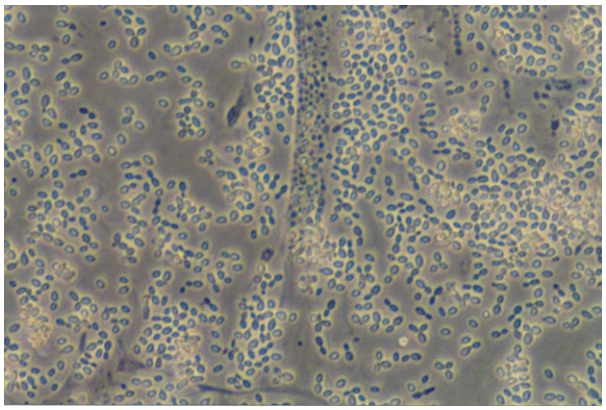

Fig. 1. Photomicrograph showing organism budding from cultured choroid plexus cells and floating in the culture medium (20 ×).

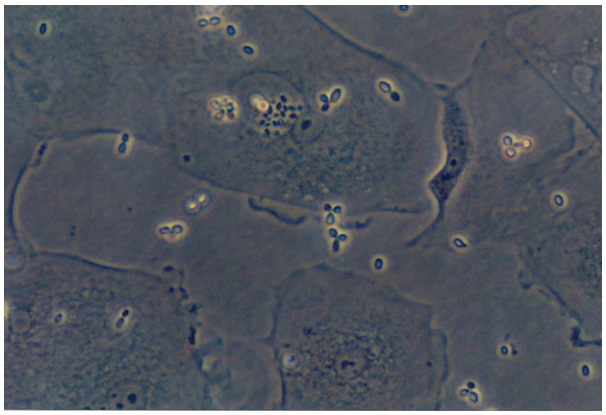

Fig. 2. Photomicrograph showing organism budding from cultured blood monocytes and floating in the culture medium (20 ×).

not previously encountered this fungus when obtaining tissues for primary cell culture, steps were taken to precisely identify the organism infecting the cells.

Serum from the lamb was submitted to the Colorado State University Veterinary Diagnostic Laboratory for fungal diagnosis and was found negative for Aspergillosis, Blastomycosis, Coccidioidomycosis and Histoplasmosis by agarose gel immunodiffusion (AGID) assay. Subsequently, DNA was isolated from fungi collected from tissue culture supernatants (ArchivePure DNA Yeast & Gram+ Kit; 5 Prime, Inc., Gaithersburg, MD, USA) and used in PCR assays for a variety of fungal pathogens (Guedes et al., 2003; Lau et al., 2007) with DNA from *Candida albicans* acting as a positive control. The PCR products were sequenced and BLAST analysis performed. Fungal samples obtained from the choroid plexus cultures were simultaneously submitted to The Fungal Testing Laboratory at The University of Texas Health Science Center at San Antonio for identification.

Results

Amplification of fungal DNA using *Histoplasma*-specific primers (Bracca et al., 2003; Guedes et al., 2003) was negative. However, when the product of a panfungal PCR assay that targets the internal transcribed spacer (ITS1) region of the ribosomal DNA cluster (Lau et al., 2007) was sequenced, the unknown fungal sequence aligned with 100% identity to *Rhodotorula minuta*. Results from the Fungal Testing Laboratory verified this identification.

Discussion

Rhodotorula sp. are emerging opportunistic pathogens, particularly in immunocompromised patients (Diekema et al., 2005; Tuon and Costa, 2008; Krzysciak and Macura, 2010; Wirth and Goldani, 2012). They have a long-oval shape, are arranged in pairs and appear pink, shiny and smooth on agar (National Collection of Yeast Cultures; http://www.ncyc.co.uk/yeast-ncyc-931.html). They have been found in the atmosphere in Japan, human sputum in the Netherlands and Germany, human throat swabs in Norway and Hungary, sea-water off Florida and Sweden, shrimp off the coast of Texas, a decaying human tooth in South Africa, a mycotic nodule in a white rat, oat leaves and the gut of a sheep in France (CBS-KNAW Fungal Biodiversity Centre; http://www.cbs.knaw.nl/collections/BioloMICS.aspx). In a review of 128 human *Rhodotorula* cases (Tuon and Costa, 2008), 79% were fungemia, 7% were eye infections and 5% were peritonitis. The most common isolated risk factor associated with infection was use of a central venous catheter, and overall mortality was 12.6%. *Rhodotorula* has also been found to cause meningitis (Gyaurgieva et al., 1996; Sundaram et al., 2006). There have been reports of *Rhodotorula* infection resolving without the use of antifungal treatment, which may suggest low virulence in immunocompetent individuals (Tuon and Costa, 2008). A recent review by Wirth and Goldani (2012) lists reports of skin infections in chickens, and a lung infection in sheep caused by *R. mucilaginosa*. Infection with *Rhodotorula* spp. was also reported in a sea lion, cats, cattle, ostriches, birds, monkeys and camels (Wirth and Goldani, 2012).

The evidence for infection in this case did not appear to be coincidental. The ewe in our study was normal upon gross examination. Budding yeast was not noticeable in cell cultures until approximately 3 weeks after isolation, indicating a low level of undetected infection. The presence of *R. minuta* in our cell cultures was not caused by contaminated medium because the same medium was used in multiple cell cultures from other animals that did not become infected. These included bovine monocyte-derived macrophages cultured in the medium containing 5% FBS, and an ovine kidney cell line, LM929 cells, and a porcine

monocyte-derived macrophage cell line cultured in the medium containing 10% FBS. All cultures were maintained in vented cap 25 or 75 cm^2 flasks. If the contamination was present in the necropsy facility, it should not have occurred in the blood cell cultures because blood was collected aseptically into sterile syringes. If the ewe had not been euthanized, it is possible that this infection would have gone unnoticed and possibly resolved as described above in some human cases. However, it is also possible that the infection may have spread and caused severe illness. We believe this to be the first report of *Rhodotorula* fungemia in a sheep in the United States. Infection with *Rhodotorula* spp. may be more widespread than recognized and the relationship of infection, immunosuppression and disease is critical in understanding emerging *Rhodotorula* infections.

Acknowledgements

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. The USDA is an equal opportunity provider and employer. The authors thank Dr. Richard McKown for PCR primer selection, and Ms. Stacy Bierman for technical assistance.

References

Bracca, A., M.E. Tosello, J.E. Girardini, S.L. Amigot, C. Gomez and E. Serra, 2003: Molecular detection of Histoplasma capsulatum var. capsulatum in human clinical samples. *J. Clin. Microbiol.* 41, 1753–1755.

Chitko-McKown, C.G. and M.D. Macneil, 2010: Development of fibroblast cell lines from the cow used to sequence the bovine genome. *Anim. Genet.* 41, 445.

Chitko-McKown, C.G., J.M. Fox, L.C. Miller, M.P. Heaton, J.L. Bono, J.E. Keen, W.M. Grosse and W.W. Laegreid, 2004: Gene expression profiling of bovine macrophages in response to *Escherichia coli* O157:H7 lipopolysaccharide. *Dev. Comp. Immunol.* 28, 635–645.

Diekema, D.J., B. Petroelje, S.A. Messer, R.J. Hollis and M.A. Pfaller, 2005: Activities of available and investigational antifungal agents against rhodotorula species. *J. Clin. Microbiol.* 43, 476–478.

Federation of Animal Science Societies, 2010: Guide for the care and use of agricultural animals in research and teaching. Available at http://www.fass.org/docs/agguide3rd/Ag_Guide_3rd_ed.pdf (accessed October 19, 2012).

Guedes, H.L., A.J. Guimaraes, M. Muniz Mde, C.V. Pizzini, A.J. Hamilton, J.M. Peralta, G.S. Jr Deepe and R.M. Zancope-Oliveira, 2003: PCR assay for identification of histoplasma capsulatum based on the nucleotide sequence of the M antigen. *J. Clin. Microbiol.* 41, 535–539.

Gyaurgieva, O.H., T.S. Bogomolova and G.I. Gorshkova, 1996: Meningitis caused by *Rhodotorula rubra* in an HIV-infected patient. *J. Med. Vet. Mycol.* 34, 357–359.

Krzysciak, P. and A.B. Macura, 2010: Drug susceptibility of 64 strains of *Rhodotorula* sp. *Wiad. Parazytol.* 56, 167–170.

Lau, A., S. Chen, T. Sorrell, D. Carter, R. Malik, P. Martin, P. Martin and C. Halliday, 2007: Development and clinical application of a panfungal PCR assay to detect and identify fungal DNA in tissue specimens. *J. Clin. Microbiol.* 45, 380–385.

Sundaram, C., P. Umabala, V. Laxmi, A.K. Purohit, V.S. Prasad, M. Panigrahi, B.P. Sahu, M.V. Sarathi, S. Kaul, R. Borghain, A.K. Meena, S.S. Jayalakshmi, A. Suvarna, S. Mohandas and J.M. Murthy, 2006: Pathology of fungal infections of the central nervous system: 17 years' experience from Southern India. *Histopathology* 49, 396–405.

Tuon, F.F. and S.F. Costa, 2008: Rhodotorula infection. A systematic review of 128 cases from literature. *Rev. Iberoam. Micol.* 25, 135–140.

Wirth, F. and L.Z. Goldani, 2012: Epidemiology of Rhodotorula: an emerging pathogen. *Interdiscip. Perspect. Infect. Dis.* 2012, 1–6.