Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Veterinary Parasitology: Regional Studies and Reports 12 (2018) 9–12

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Case report

Respiratory disease associated with migrating *Ascaris* larvae in a beef calf

Jeba Jesudoss Chelladurai[a], Rachel Derscheid[b], Matthew T. Brewer[a,*]

[a] Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

ABSTRACT

A group of 4-month-old beef calves were examined for clinical respiratory disease with labored breathing, coughing, and fevers of over 104 °F. Necropsy of one of the calves revealed lungs that were not collapsed but had red mottled appearance on cut surface. Assessment of lung tissue by bacterial culture and PCR did not reveal bovine bacterial or viral respiratory pathogens. Histopathology of affected tissues and lymph nodes revealed larval ascarid nematodes. In combination with phylogenetic analysis, amplification and sequencing of ITS1 was used to identify the larvae as *Ascaris*.

1. Introduction

Differential diagnoses for parasitic pneumonia in cattle in North America are infection with the trichostrongyle lungworm *Dictyocaulus viviparus* (Panuska, 2006) and larvae of the ascarids - *Toxocara vitulorum* and *Ascaris* spp. (Bowman and Georgi 2017). *Dictyocaulus* infections are known to occur sporadically in the Midwest, including Iowa (Al-Qudah et al., 1995). Dictyocaulosis can be diagnosed by (a) demonstrating the slender, white adult worms in the bronchi of the caudal lobes in gross specimens at necropsy, (b) visualizing prominently alate adult worms with coelomyarian musculature and embryonated egg in the uterus in bronchi by histopathological techniques, or (c) through the recovery of larva using the Baermann technique (Al-Qudah et al., 1995). However, confirmatory diagnosis of ascarid larval infections by classical pathological and parasitological techniques is difficult, without additional molecular techniques because (a) complete sets of microanatomical measurements and descriptions of only a few ascarid larvae in mice tissue are available (Bowman, 1987), and it is unknown if the larvae measure the same in cattle lungs (b) ascarid larvae undergo changes in length and width to different extents during the migratory phase (Nichols, 1956a).

Cattle are the natural definitive hosts for the ascarid *Toxocara vitulorum*, which follows a typical hepato-pulmonary- tracheal migratory route and therefore larvae of this species can be demonstrated in the liver, lung, and intestine of an infected bovine (Roberts, 1990). Past reports of pulmonary pneumonia associated with migration of *Ascaris* spp. in cattle have been documented in experimental infections and situations where cattle had a known history of being housed in buildings/paddocks contaminated with pig feces (McCraw and Greenway, 1970; McCraw and Lautenslager, 1971; Morrow, 1968).

While migrating *T. vitulorum* larvae in cattle lungs in non-endemic areas may represent an outbreak in the herd (Chelladurai et al., 2015), cattle are only accidental hosts for *Ascaris* and worms do not mature to the adult stage. Given the different approaches for managing each disease at the herd level, it is important to differentiate between the larval ascarids using molecular techniques such as PCR. In this report, we demonstrate the utility of combining histopathology and PCR to identify ascarid larvae associated with respiratory disease in beef calves.

2. Materials and methods

2.1. Necropsy and histopathology

A field necropsy was conducted on an approximately 4-month-old calf. The referring veterinarian submitted fresh heart, lung, intestine, ear notch, and lymph node to Iowa State Veterinary Diagnostic Laboratory. Upon arrival, sections of lung, heart, lymph node, intestine, and ear notch were fixed in 10% neutral-buffered formalin, processed for histopathology using routine methods, and embedded in paraffin. Four-micron thick sections were stained with hematoxylin and eosin and the slides were examined by a board-certified veterinary pathologist (RD). All methods were performed in accordance with the University Institutional Animal Use and Care committee as well as local laws.

---

* Corresponding author at: 2758 Vet Med, 1800 Christensen Dr., Ames, IA 50011, USA
E-mail address: brewermt@iastate.edu (M.T. Brewer).

https://doi.org/10.1016/j.vprsr.2017.12.005
Received 8 November 2017; Accepted 24 December 2017
Available online 27 December 2017
2405-9390/ © 2018 Elsevier B.V. All rights reserved.

2.2. Molecular studies

Total genomic DNA was extracted from the formalin-fixed, paraffin-embedded lung tissue, using MagMAX FFPE DNA Isolation Kit (Life Technologies, Grand Island, NY) according to the manufacturer's protocol. DNA was eluted into 100 µl of nuclease free water and stored at − 20° C.

PCR Amplification of the partial Internal Transcribed Spacer 1 gene was carried out in 24 µl reactions, with 1 µl of genomic DNA, 1 × PCR buffer, 3 mM $MgCl_2$, 0.25 µM of each dNTP, 0.5 µM of each primer (5’-CCG GGC AAA AGT CGT AAC AA-3′ and 5′–TAG TGC TCA ATG TGT CTG CA-3′) and 2 units of *Taq* polymerase per reaction (Promega, Madison, WI) (Arizono et al., 2010). PCR products were sequenced twice in both directions on an Applied Biosystems 3730xl DNA analyzer at the Iowa State University DNA Facility, and assembled with the CAP3 sequence assembly program (Huang and Madan, 1999). Sequences were compared with those in the NCBI GenBank database using nucleotide BLAST.

Multiple alignments of nucleotide sequence data were made using ClustalW and trimmed to 360 nt on MEGA7 (Kumar et al., 2016). Best model fit for nucleotide substitutions was selected using jModeltest 2.1.10 (Darriba et al., 2012). Corrected Akaike Information Criterion (AICc) calculations were used to select the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) with a discrete gamma distribution (HKY + G) as the best fit model for Maximum Likelihood tree construction using MEGA7.

3. Results

3.1. Necropsy and histopathology

A necropsy was performed by the referring veterinarian who submitted tissues to the Iowa State University Veterinary Diagnostic Lab for evaluation. Grossly, the lungs had a slightly non-collapsed appearance and mild red mottling on cut surface. Formalin-fixed tissues were embedded in paraffin and processed

Within the lung, bronchi and bronchioles were frequently ectatic and contained fibrin admixed with degenerate neutrophils and had many 50-90 µm diameter nematode larvae enmeshed within the exudate (Figs. 1, 2). Epithelium of bronchi and bronchioles was variably attenuated or hyperplastic with loss of cilia. Airways were cuffed by lymphocytes and plasma cells. Surrounding lobules were partially atelectatic and alveoli were filled by high-protein edema and scant fibrin. The interstitium was variably, mildly expanded by lymphocytes and fibrin. Larvae had coelomyarian, polymyarian musculature, prominent lateral alae, and large lateral chords. Intestine of larvae was lined by cuboidal to low columnar, uninucleate cells (Fig. 3). Within an associated lymph node there were rare nematode larvae within the parenchyma or subcapsular sinus that were surrounded by macrophages and fewer neutrophils.

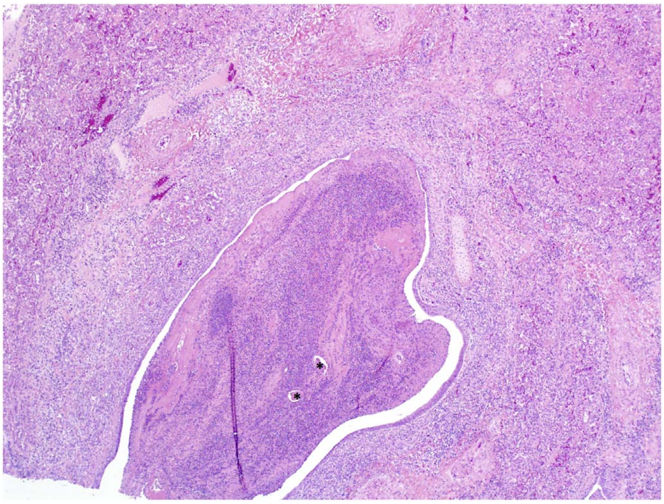

Fig. 1. An ectatic bronchus filled with cellular debris, degenerate neutrophils, and fibrin. There is segmental attenuation of epithelium and a mononuclear infiltrate in the adventitia. Asterisks indicate larval nematodes. (40 × magnification).

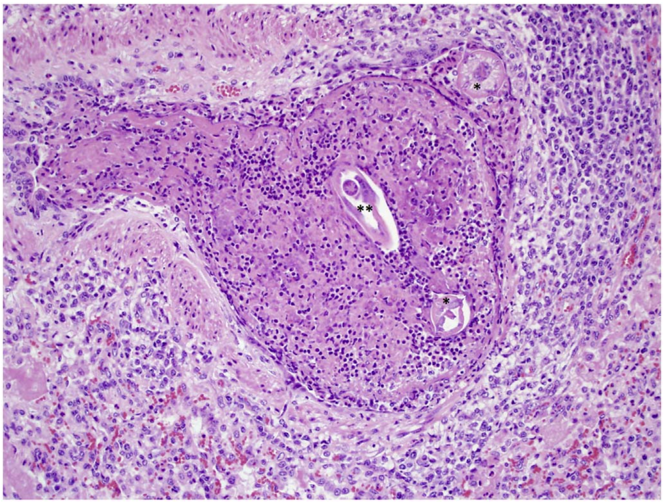

Fig. 2. Bronchioles are distended by degenerate neutrophils, cellular debris, and fibrin with multifocal ascarid larvae in cross (*) and tangential (**) section. Epithelium is attenuated and there is smooth muscle hyperplasia. Plasma cells and lymphocytes expand the adventitia (200 × magnification).

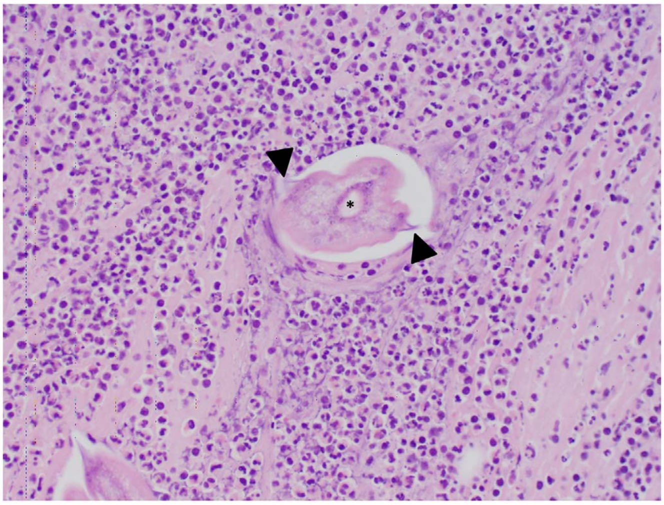

Fig. 3. Larval morphology evident in this section includes prominent lateral alae (arrowheads), coelomyarian, polymyarian musculature, and intestine(*) lined by a single layer of cuboidal epithelium. (400 × magnification).

3.2. Ancillary test results

Lung tissue had no growth on routine bacterial culture and PCR testing for *Mycoplasma bovis, Mannehimia haemolytica, Histophilus somni,* and *Pasteurella multocida* were negative. Both PCR and immunohistochemistry were negative on lung tissue for bovine corona virus, bovine respiratory syncytial virus, and infectious bovine rhinitracheitis virus (Bovine Herpesvirus-1). Bovine viral diarrhea virus PCR of lung tissue was negative, as was immunohistochemistry on ear notch. No parasites were observed on fecal flotation.

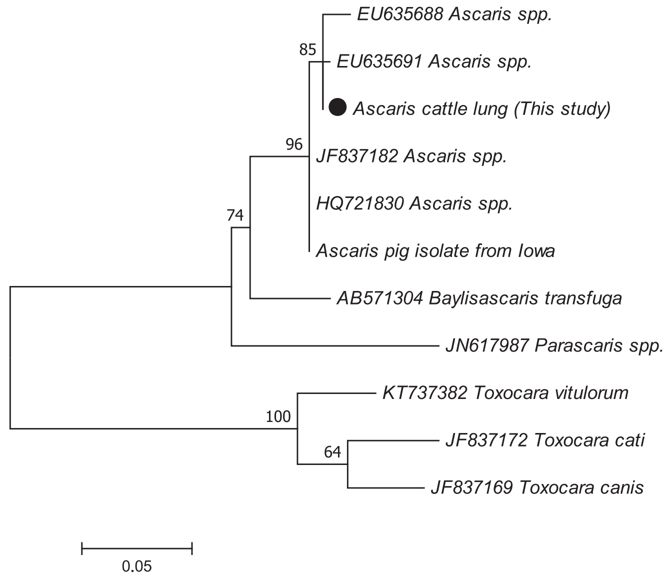

Fig. 4. Phylogeny was inferred by using the Maximum Likelihood method. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The larval nematodes recovered in this study cluster closely with *Ascaris*.

3.3. Sequencing results

PCR amplification of the partial Internal Transcribed Spacer 1 gene was carried out on DNA extracted from formalin fixed paraffin embedded lung tissue, sequenced on an ABI 3730xl DNA Analyzer at the Iowa State University DNA facility, and assembled using CAP3 (available at http://doua.prabi.fr/software/cap3). Assembled sequences were compared to sequences on GenBank using nucleotide BLAST. A 368 nt fragment of the partial ITS1 gene was obtained after sequencing. Nucleotide BLAST revealed that the sequence had 100% identity with several isolates of *Ascaris spp.* (KY576142, KY576143, KU522455, AB576588, AB571295, EF153619-EF153623) and 82–84% identity with *Toxocara vitulorum* (KT737382, KJ777180-KJ777182). PCR amplification of the mitochondrial cytochrome oxidase I (cox1) and nuclear SSU (18S) gene using the FFPE DNA template failed.

3.4. Phylogenetic analysis

To demonstrate the phylogenetic relationship between the migrating ascarid larvae and other ascarids, a Maximum Likelihood tree was constructed (Fig. 4). A pig isolate from a previous study that had a 3 band PCR-RFLP pattern (ITS PCR amplicons digested with *Hae*III) from the state of Iowa, USA was included in the analysis (Jesudoss Chelladurai et al., 2017).

Partial *Ascaris* ITS1 gene sequenced in this study was found to cluster with the partial ITS1 gene from other *Ascaris* isolates. In contrast, *Toxocara vitulorum*, *Toxocara cati* and *Toxocara canis* occur in a distinctly separated cluster.

4. Discussion

Pneumonia associated with migrating *Ascaris* spp. has been described in cattle (McCraw and Greenway, 1970; McCraw and Lautenslager, 1971; Morrow, 1968), based solely on larval morphology in tissue sections, and larval recovery from the lung using the Baermann method. However, it is difficult to differentiate the larvae of ascarids in histological sections. This report details the use of molecular techniques for the identification of *Ascaris* in formalin fixed paraffin embedded (FFPE) tissue from the lung of a calf, to support gross and histopathological diagnoses.

Compared with mild gross lesions, histopathology revealed more serious inflammation associated with larvae migrating in the lung. Characteristics of the larvae included alae, coelomyarin musculature, prominent lateral chords, and an intestine lined by uninucleate cells. Morphological features allowed for preliminary identification of the larvae as an ascarid larvae (Nichols, 1956b). An acute to subacute bronchiolitis and interstitial pattern was observed similar to the lesions observed by McCraw (McCraw and Lautenslager, 1971). Contrary to the findings described by McCraw and Leuternslager, gross lesions were fairly subtle in this case. Follow-up on the outcome of the disease in the remaining cohort of calves was unavailable.

Larval migratory patterns following the ingestion of infective egg and hatching in the alimentary tract are similar among members of the ascarids. A hepatic-pulmonary-tracheal route is followed by *Ascaris* in its definitive (Douvres et al., 1969) and accidental hosts including humans and cattle (Kennedy, 1954; Nichols, 1956b). *Toxocara vitulorum*, the cattle ascarid, must be considered in the differential diagnosis, because it follows a similar hepatic-pulmonary-tracheal route (Warren, 1971), infects calves, and has been recently reported in North America (Chelladurai et al., 2015; Davila et al., 2010; Woodbury et al., 2012). Therefore, molecular techniques are essential for obtaining a definitive diagnosis. Adults of the *Dictylocaulus viviparus* which reside in the bronchi are also capable of causing parasitic bronchitis (Panuska, 2006).

The use of polymerase chain reaction (PCR) to amplify the multi-copy, nuclear internal transcribed spacer region (ITS) that occurs between the SSU and LSU ribosomal DNA for the identification of ascarid larvae has been described (Ishiwata et al., 2004). Although this approach is specific and sensitive enough to differentiate between *Dictyocaulus viviparus*, *Toxocara vitulorum* and *Ascaris spp.*, neither this method nor PCR-RFLP of the ITS gene can be used to differentiate between pig and human isolates of *Ascaris* (Nejsum et al., 2017). But, this approach is still useful to draw comparisons and establish a preliminary population epidemiology between isolates from a given area and from across the world (Chelladurai and Brewer, 2017). Given the propensity of *Ascaris* for cross transmission between animals and humans (Anderson, 1995), and the very high identity of amino acid sequences encoded by the mitochondrial genome in human and pig isolates (Liu et al., 2012), the debate on the existence of one or two species continues. The limitations of the use of the ITS gene and difficulty in amplifying other genes from FFPE tissue precludes identification of the *Ascaris* larvae in this study as being from a pig or human source.

In this case, we characterized a case of pneumonia in beef calves that was solely associated with *Ascaris* larvae, while no other viral or bacterial agents were detected. There are no products labeled for the treatment of *Ascaris* larvae in cattle, but removing cattle from bedding or soil contaminated with swine feces may decrease future clinical signs. In this case, there was no known exposure to swine feces. Because ascarid ova can live for years in soil, the incriminated soil or bedding should be removed from cattle lots to prevent future cases.

Ethics

The authors certify that we complied with all local laws and Iowa State University IACUC rules while conducting this research.

References

Al-Qudah, K., Greve, J.H., Wass, W.M., 1995. Dictyocaulus Viviparus in an Iowa dairy cow. Iowa State Univ. Vet. 57, 10–13.

Anderson, T.J., 1995. Ascaris infections in humans from North America: molecular evidence for cross-infection. Parasitology 110 (Pt 2), 215–219.

Arizono, N., Yoshimura, Y., Tohzaka, N., Yamada, M., Tegoshi, T., Onishi, K., Uchikawa, R., 2010. Ascariasis in Japan: is pig-derived Ascaris infecting humans? Jpn. J. Infect. Dis. 63, 447–448.

Bowman, D.D., 1987. Diagnostic morphology of four larval ascaridoid nematodes that may cause visceral larva migrans: Toxascaris leonina, Baylisascaris procyonis, Lagochilascaris sprenti, and Hexametra leidyi. J. Parasitol. 73, 1198–1215.

Bowman, D.D., Georgi, J.R., 2017. Georgis' Parasitology for Veterinarians, 10th edition.

(Edition, xi, 477 pages pp).

Chelladurai, J.J., Brewer, M.T., 2017. Reply to Nejsum et al. J. Infect. Dis. 216, 617–618.

Chelladurai, J.J., Bader, C., Snobl, T., Magstadt, D., Cooper, V., Brewer, M.T., 2015. Toxocara vitulorum infection in a cohort of beef calves in Iowa. Vet. Parasitol. 214, 96–99.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Davila, G., Irsik, M., Greiner, E.C., 2010. Toxocara vitulorum in beef calves in North Central Florida. Vet. Parasitol. 168, 261–263.

Douvres, F.W., Tromba, F.G., Malakatis, G.M., 1969. Morphogenesis and migration of Ascaris suum larvae developing to fourth stage in swine. J. Parasitol. 55, 689–712.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.

Huang, X.Q., Madan, A., 1999. CAP3: a DNA sequence assembly program. Genome Res. 9, 868–877.

Ishiwata, K., Shinohara, A., Yagi, K., Horii, Y., Tsuchiya, K., Nawa, Y., 2004. Identification of tissue-embedded ascarid larvae by ribosomal DNA sequencing. Parasitol. Res. 92, 50–52.

Jesudoss Chelladurai, J., Murphy, K., Snobl, T., Bader, C., West, C., Thompson, K., Brewer, M.T., 2017. Molecular epidemiology of Ascaris infection among pigs in Iowa. J. Infect. Dis. 215, 131–138.

Kennedy, P.C., 1954. The migrations of the larvae of Ascaris lumbricoides in cattle and their relation to eosinophilic granulomas. Cornell. Vet. 44, 531–565.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Liu, G.H., Wu, C.Y., Song, H.Q., Wei, S.J., Xu, M.J., Lin, R.Q., Zhao, G.H., Huang, S.Y., Zhu, X.Q., 2012. Comparative analyses of the complete mitochondrial genomes of Ascaris Lumbricoides and Ascaris suum from humans and pigs. Gene 492, 110–116.

McCraw, B.M., Greenway, J.A., 1970. Ascaris suum infection in calves. 3. Pathology. Can. J. Comp. Med. 34, 247–255.

McCraw, B.M., Lautenslager, J.P., 1971. Pneumonia in calves associated with migrating Ascaris suum larvae. Can. Vet. J. 12, 87–90.

Morrow, D.A., 1968. Pneumonia in cattle due to migrating Ascaris lumbricoides larvae. J. Am. Vet. Med. Assoc. 153, 184–189.

Nejsum, P., Betson, M., Stothard, R., 2017. Analysis of ribosomal DNA cannot unequivocally assign Ascaris to species level or identify hybrids. J. Infect. Dis. 216, 616–617.

Nichols, R.L., 1956a. The etiology of visceral larva migrans. I. Diagnostic morphology of infective second-stage Toxocara larvae. J. Parasitol. 42, 349–362.

Nichols, R.L., 1956b. The etiology of visceral larva migrans. II. Comparative larval morphology of Ascaris Lumbricoides, Necator Americanus, Strongyloides stercoralis and Ancylostoma caninum. J. Parasitol. 42, 363–399.

Panuska, C., 2006. Lungworms of ruminants. Vet. Clin. North Am. Food Anim. Pract. 22, 583–593.

Roberts, J.A., 1990. The life cycle of Toxocara Vitulorum in Asian buffalo (Bubalus Bubalis). Int. J. Parasitol. 20, 833–840.

Warren, E.G., 1971. Observations on the migration and development of Toxocara Vitulorum in natural and experimental hosts. Int. J. Parasitol. 1, 85–99.

Woodbury, M.R., Copeland, S., Wagner, B., Fernando, C., Hill, J.E., Clemence, C., 2012. Toxocara Vitulorum in a bison (Bison Bison) herd from western Canada. Can. Vet. J.-Revue Vet. Can. 53, 791–794.