



NOTE

Wildlife Science

Hematological and pathological features of massive hepatic necrosis in two radiated tortoises (*Astrochelys radiata*)

Nobuhide KIDO^{1)*}, Iori ITAGAKI²⁾, Daisuke KIRYU¹⁾, Tomoko OMIYA¹⁾ and Kaori ONO¹⁾

¹⁾Nogeyama Zoological Gardens, Yokohama Greenery Foundation, 63-10, Oimatsu cho, Nishi ku, Yokohama, Kanagawa 220-0032, Japan

²⁾The corporation for production and research of laboratory primates, 1-16-2, Sakura, Tsukuba shi, Ibaraki 305-0003, Japan

J. Vet. Med. Sci.

79(1): 166–170, 2017

doi: 10.1292/jvms.16-0142

Received: 21 March 2016

Accepted: 4 October 2016

Published online in J-STAGE:
16 October 2016

ABSTRACT. Two radiated tortoises (*Astrochelys radiata*) exhibited anorexia and hypokinesia. In both cases, hematological and serum biochemical examinations revealed high alkaline phosphatase levels, moderately high aspartate aminotransferase levels and white blood cell counts approximately within the normal range. Despite being treated, the tortoises died 9 and 43 days after the first clinical examination. Gross pathological examinations revealed that the livers of both animals were extremely swollen and contained pale yellow necrotic tissue. Histopathological assessment revealed that the livers contained a massive area of hepatic necrosis surrounded by migration of macrophages and multinucleated giant cells. In one of the cases, severe fibrosis was observed. The present study provides reference information for similar cases in the future.

KEY WORDS: *Astrochelys radiata*, massive hepatic necrosis, radiated tortoise, serum biochemical value

A female radiated tortoise (case 1; body weight, 7.0 kg; age unknown and carapace length, 33.0 cm) that originally inhabited the southern part of Madagascar [1] was brought to the Nogeyama Zoological Gardens, because of a violation of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The tortoise was maintained for 5 years and 5 months in the same space with 7 other female radiated tortoises. The tortoises were always in contact with each other. The enclosure had concrete flooring and air-conditioning to regulate humidity and temperature. In the first case, the tortoise stopped eating food for several days before the medical examination. A radiographic examination did not reveal any abnormal signs. The tortoise was administered a ceftazidime injection (20 mg/kg; Mylan Seiyaku, Osaka, Japan) every 72 hr. The tortoise recovered its appetite on day 16, at which point the antibiotic administration was discontinued. However, subsequently, the tortoise appeared debilitated due to hypokinesia and anorexia on day 29. The hematological and serum biochemical examinations revealed high aspartate aminotransferase (AST) and moderately low packed cell volume (PCV) values (Table 1). Therefore, a liver disorder was considered. Multi-vitamins (Rebationin; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), tiopronin (Dobutuyou Tiora injection, Aska Pharmaceutical Co., Ltd., Tokyo, Japan) and glutathione (Tachion injection; Teva Pharma Japan Inc., Nagoya, Japan) were subcutaneously administered with normal saline for five days, with the aim of recovering liver function. In addition, ceftazidime administration was reinitiated. However, the tortoise appeared severely debilitated until day 42, and hematological and serum biochemical examinations revealed a high white blood cell (WBC) count, and high levels of AST, alkaline phosphatase (ALP), blood urea nitrogen (BUN) and uric acid (UA) (Table 1). The tortoise eventually died on day 43.

A gross pathological examination of the tortoise revealed that the liver was extremely swollen, hard and contained a massive area of pale yellowish necrotic tissue (Fig. 1a). In addition, an abscess was found in the kidney. The whole lung appeared hyperemic, and the trachea contained foamy liquid. The heart, lung, liver, stomach, intestine, kidney and spleen were collected and stored in 20% neutral buffered formalin solution. Histopathological examination (Marupi Lifetech Co., Ltd., Osaka, Japan) revealed massive hepatic necrosis with heterophil infiltration after hematoxylin and eosin (H&E) staining. Abundant macrophages and associated multinuclear giant cells surrounded the necrotic tissue. Acid-fast bacteria and fungi were not detected after Ziehl-Neelsen and periodic acid-Schiff (PAS) staining. No related histopathological changes were found in other organs and tissues, except for mild heterophilic, macrophagial and lymphocytic infiltrations of the bronchi. Minimal fibrin thrombi were observed in several pulmonary veins, and renal calcinosis was observed.

*Correspondence to: Kido, N., Kanazawa Zoological Gardens, 5-15-1, Kamariya higashi, Kanazawa ku, Yokohama, Kanagawa 236-0042, Japan. e-mail: kido@hama-midorinokyokai.or.jp

©2017 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/4.0/>>.

Table 1. The hematological and serum biochemical values of the radiated tortoise in cases 1 and 2

		Case 1		Case 2				Reference 1	Reference 2	
		Ex. Day	Day 29	Day 42	Day 1	Day 3	Day 4			Day 7
Ht	//		0.14	0.14	0.12	0.11	0.1	0.08	0.1–0.51	0.31 ± 0.0739
Hb	g//			59	34	39	33	20	40–80	67 ± 15.1
RBC	10 ¹² //		0.4	0.33	0.6	0.33			0.3–1.1	0.5 ± 0.115
WBC	10 ⁹ //		7.92	22.44	5.5	3.96			2.5–14	4.3 ± 1.0
Baso	10 ⁹ //		0	0	0				0.1–2.5	
Eo	10 ⁹ //		0	0	0				0.03–0.82	
He	10 ⁹ //		6.73	17.73	3.41				0.7–8.0	
Lym	10 ⁹ //		1.19	4.49	2.09				0.4–5.8	
Mo	10 ⁹ //		0	0	0				0.02–0.5	
TP	g//		53	66	29	26	22	20		39.7 ± 4.52
ALB	g//		22	38	9	7	7	6		11 ± 1.4
AST	IU//		403	961	249	253	219	230	25–348	72.7 ± 24.58
ALP	IU//			2009	806	670	608	524	72–392	92.7 ± 14.3
LDH	IU//				851	>900				401.8 ± 121.12
GLU	mmol//		2.66	8.44	0.11	1	2.94			3.319 ± 0.7061
BUN	mmol//			32.31	17.46	30.24	33.63	42.59	0.714–12.138	
TBIL	umol//			25.65	8.55	13.68			0–8.55	
TCHO	mmol//			2.17	0.34	0.57	0.59	0.59	1.448–3.982	2.715 ± 0.671
Ca	mmol//		2.4	3.34	2.2	2.42	2.52	2.67	2.146–4.491	3.044 ± 0.227
UA	umol//		136.8	368.78	214.13	362.83	422.31	565.06		16.654 ± 11.539
TG	mmol//			>5.65	0.01	0.01		0.01		
IP	mmol//			1.42	0.84	0.61	0.65	0.39		1.030 ± 0.147
Mg	mmol//			2.06	1.52	1.6				
Na	mmol//			114	126	124	121	117		126.8 ± 3.34
K	mmol//			3.4	3.2	3.6	3.8	3.8		5.5 ± 0.24
Cl	mmol//			81	95	96	94	87	91–112	96.5 ± 2.69

Reference 1: [2]. Reference 2: [9].

Another female radiated tortoise (*Astrochelys radiata*; case 2: weight, 3.0 kg; age, unknown and carapace length, 27.6 cm) was brought to the NogeYama Zoological Gardens, Japan, also because of a violation of the CITES. This second tortoise was maintained for 7 years with 7 other female radiated tortoises, including the tortoise in case 1. All 7 tortoises always remained in contact with each other. Soon after the tortoise in case 1 died, this female tortoise exhibited hypokinesia for about 1 month and appeared anorexic a few days before a medical examination. On the first day of the examination, blood samples for hematological and serum biochemical examinations were collected from the saphenous vein. The examinations revealed a low PCV, low hemoglobin and red blood cell counts, high AST, ALP, BUN and UA values, and low total protein and albumin values (Table 1). A radiographic examination revealed a large mass with a moderately high opacity in the upper middle area of the body, and that occupied half of the body area. A diagnosis of a liver disorder was considered, and the tortoise was administered drip injections, including Ringer solution (Ringeru V with vitamin B1; Nippon Zenyaku Kogyo Co., Ltd.), multi-vitamins, glycyrrhizic ammonium (Stronger NEO-minophagen C injection; Minophagen Pharmaceutical Co., Ltd., Tokyo, Japan), dopamine hydrochloride (Dominin Tenteki injection; Nippon Shinyaku Co., Ltd., Kyoto, Japan), iron (Fejin injection; Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan) and cefazolin sodium (Rasenazorin; Nichi-Iko Pharmaceutical Co., Ltd.). Hematological and serum biochemical analyses were also performed on days 3, 4 and 7. Although the ALP value slightly decreased, the BUN and UA values increased, and all other values showed no improvement (Table 1). The tortoise in case 2 exhibited edema in the extremities on day 6 and died on day 9.

Gross pathological examination revealed an overall hardening of the right lobe of the liver and the presence of pale yellow, caseous and necrotic tissue in half of the right lobe (Fig. 1b). We also observed the adhesion of the right lobe of the liver to a part of the small intestine. Subcutaneous and pulmonary edema and ascites were observed. Scattered white nodules were observed on the spleen. The heart, lung, liver, stomach, kidney and spleen were collected and stored in 20% neutral buffered formalin solution. Histopathological examination based on H&E staining (The Corporation for Production and Research of Laboratory Primates, Tsukuba, Japan) revealed a massive area of centrilobular hepatic necrosis that occasionally included bacterial masses of large bacilli (Fig. 2). The lesion consisted of macrophage accumulation with multinucleated giant cells and lymphoplasmacytic cell infiltration, surrounded by severe fibrosis (Fig. 3). The remaining hepatocytes were atrophic, and hemosiderin was deposited on the tissue. Bacterial masses similar to those found in the liver were also detected in the spleen, lung, heart, stomach and kidney. Multifocal necrosis was found in the spleen, stomach and kidney. In the lungs, the alveolar wall thickened with edema. In the heart, moderate edematous epicarditis with lymphoplasmacytic infiltration and minimal hemorrhagic foci was observed.

The present study reveals the clinical, hematological, serum biochemical and histopathological findings in two radiated tortoises

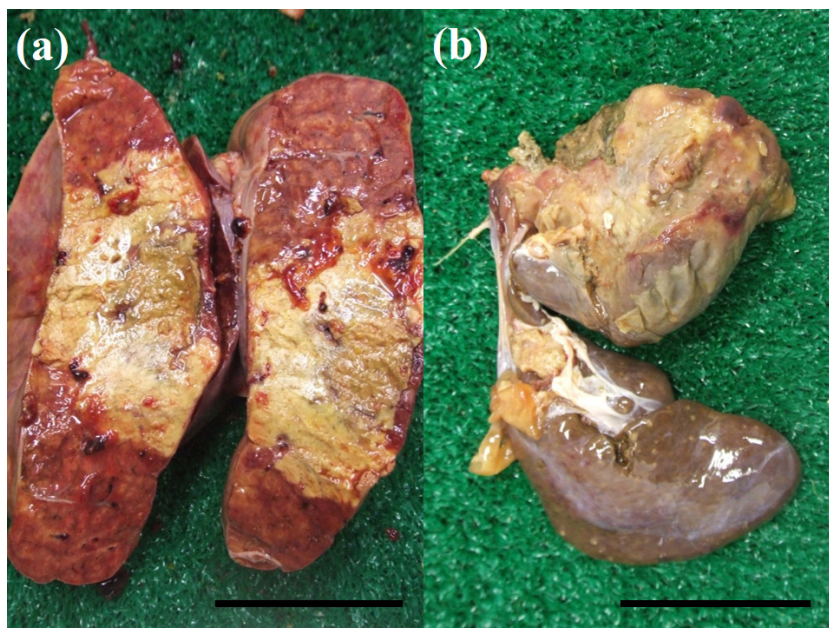


Fig. 1. Gross pathological view of the massive hepatic necrotic tissue in the radiated tortoise. (a) Case 1: The section of the liver occupied by a massive area of pale yellowish necrotic tissue. (b) Case 2: Overall hardening of the right lobe of the liver, and the presence of pale yellow necrotic tissue in half of the right lobe. Bar, 5 cm.

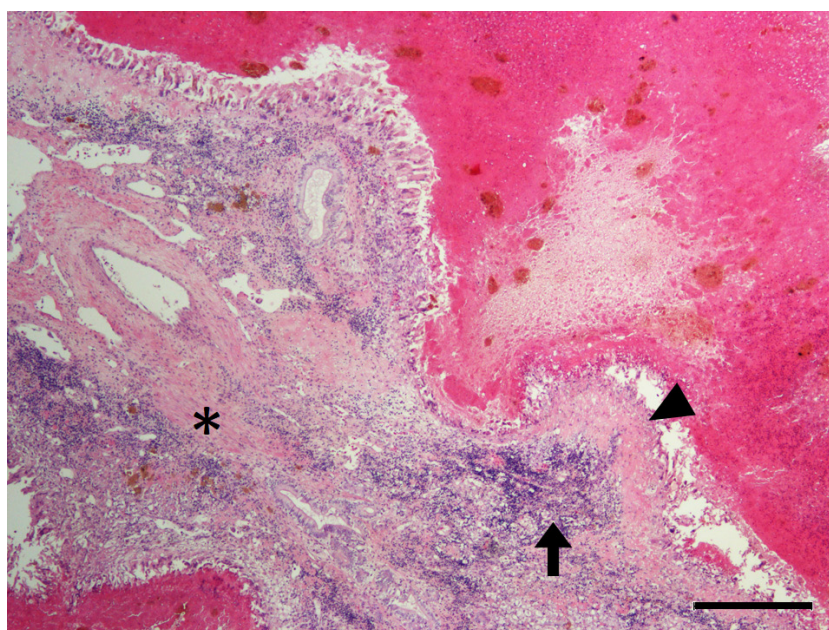


Fig. 2. Histopathological view of the massive hepatic necrotic tissue in the radiated tortoise. A massive area of hepatic necrosis and layered colliquative necrosis (arrowhead) can be observed in the liver. The infiltration of heterophils, plasma cells and lymphocytes is shown (arrow). The fibrous tissue is thickened (asterisk). Hematoxylin and eosin (H&E) staining. Bar, 500 μ m.

with massive hepatic necrosis. Both tortoises showed almost identical pathological findings in the liver. The liver was extremely swollen and occupied by pale yellow necrotic tissue. The histopathological examination revealed massive hepatic necrosis in both cases. Our findings suggest that two different types of lesions were found in both tortoises. In case 2, the necrotic lesion was surrounded by severe fibrosis, and the fibrosis hardened the liver. This observation indicates that the lesion in case 2 was more developed than that in case 1. In addition, lesions associated with septicemia were detected in case 2, because multifocal bacterial masses were observed in several systemic organs, including the lungs, heart, spleen, stomach and kidney; however, these masses were not observed in case 1. Infection might have contributed to lesion development in both cases [4, 7]. One reason for these

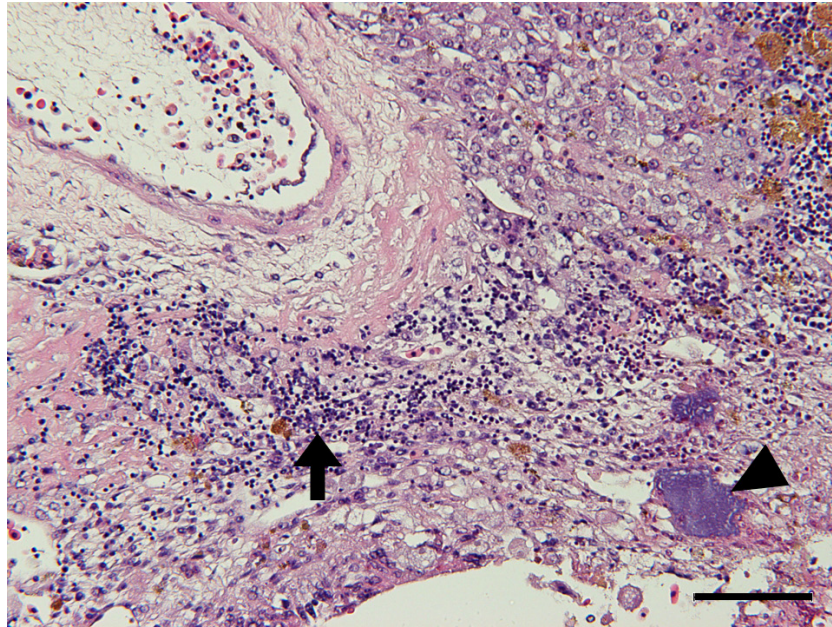


Fig. 3. Inflammation in the periportal region. The infiltration of lymphocytes and plasma cells is shown (arrow). A bacterial mass can be observed (arrowhead). Hematoxylin and eosin (H&E) staining. Bar, 100 μ m.

differences is that both tortoises received different types of antibiotics. In addition, the antibiotic treatment in case 1 probably began at an earlier stage of the disorder, i.e., soon after the symptoms manifested, than that in case 2, which began about 1 month after the presentation of hypokinesia. Therefore, the pathogen in case 1 might have been eradicated by the antibiotic treatment, thereby improving the symptoms (anorexia), albeit temporarily. Early treatment might have inhibited the progress of the lesion in case 1, whereas the lesion in case 2 was more developed than that in case 1. However, the liver lesion in case 1 was extremely severe and irreversible. Therefore, the tortoise in case 1 might have died due to the liver lesion rather than the septicemia.

The deaths of the two radiated tortoises in the present study occurred within 1.5 months after symptom onset. The two tortoises were maintained in the same area before manifesting symptoms and were in constant contact with each other. Therefore, the same pathogen might have contributed to the development of the disease. The other tortoises that were maintained in the same area were at risk of developing the infection and were carefully monitored. The histopathological examination provided useful information about the route of infection. In case 2, the most common location of the hepatocytic necrosis foci was the periportal area. In addition, bacterial masses and multifocal necrosis were observed in the stomach. These findings suggest that the infection hematogenously spread from the digestive tract. The bacillus might have then spread in the liver and caused a massive amount of necrosis, subsequently spreading to other parts of the body and inducing septicemia. Although the pathogen was not identified, and histopathological features differed between the two cases, the lesions might have been induced by the same pathogen, because these tortoises were maintained in the same area for five years, and their symptoms appeared during the same period.

Amoebiasis and adenovirus infection induce hepatic necrosis in various tortoises [6, 8, 10]. In amoebiasis, the most common lesion presented as a thickened and edematous duodenum or an ulcerated colon with a green pseudomembrane [6, 8]. In addition, amoebic trophozoites were observed in many blood vessels in these cases. The lesion in the liver exhibited multifocal to diffuse areas of necrosis. In adenovirus infection, multifocal to diffuse, hemorrhagic and necrotizing enterocolitis is frequently observed in the intestine [10]. In addition, intranuclear inclusions are identified in various organs. The lesion in the liver was an individual hepatocellular necrosis or a multifocal to submassive coagulative hepatocellular necrosis. In contrast, granulomatous hepatitis, which is common in reptiles and birds and caused by *Salmonella typhimurium* infection, was previously reported in a spur-thighed tortoise (*Testudo graeca*). In addition, numerous foci of necrosis surrounded by infiltrations of mononuclear cells were observed in the liver [3, 5]. However, in the present study, a huge necrotic area and infiltration of various inflammatory cells, including heterophils, plasma cells, lymphocytes and multinuclear cells were observed. Therefore, the characteristic lesion in the present study was unique and different from those in previous reports, although the pathogen was not detected.

Generally, disorders should be quickly identified, and treatment should be initiated at an early disease stage. Nevertheless, the tortoises presented in our cases did not exhibit any abnormal clinical signs until their lesions became untreatable. Diseased chelonians do not typically exhibit any specific symptoms other than anorexia or hypokinesia, which were the only symptoms identified in the present study. Moreover, diagnostic imaging techniques, including radiography and ultrasonography, cannot obtain specific findings for diseases in the internal organs, because of the animals' thick carapace. Although radiography revealed an unclear body obstacle in the tortoise in case 2, this observation was not sufficient to inform a definitive diagnosis. Although endoscopy and biopsy are required for the diagnosis of liver disease, the anesthesia required in these procedures posed a high but avoidable risk of deteriorating the condition of the tortoises. In contrast, hematological and serum biochemical examinations

do not require any specific materials or techniques, and serve as an index to diagnose the disease. In the present study, the hematological and serum biochemical examinations revealed that a moderately high AST value might be a key factor to detect liver disease. However, these values did not reflect the development of the lesion, because the morphological changes of the liver were extremely severe in comparison to that indicated by the serum biochemical values. Although a high ALP value was observed, this finding does not specify the extent of the liver disease [11], and the reason for the high value was unclear. The BUN and UA values increased in both tortoises. This finding might indicate dehydration or that the metabolization of BUN and UA in the kidney was affected by circulatory insufficiency or calcinosis in case 1 and multifocal necrosis in case 2 [11]. Consequently, the present study revealed that the hematological and serum biochemical features of necrotic liver disease might be uncertain and that these hematological examinations are insufficient to detect the disorder. High ALP and moderately high AST values might be the only key indicative factors of liver disease. Further studies are required to detect necrotic lesions of the liver at an early stage.

The present study provides useful information for a differential diagnosis when similar cases are encountered.

REFERENCES

1. EMYSsystem Species Page: *Geochelone radiata*. <http://emys.geo.orst.edu/collection/species/Geocheloneradiata/Geocheloneradiata.html>.
2. Gibbons, P. M., Klaphake, E. and Carpenter, J. W. 2013. Reptile. pp. 83–182. *In: Exotic animal formulary*, 4th ed. (Carpenter, J.W. ed.), Elsevier Saunders, St. Louis.
3. González Candela, M., Martín Atance, P., Seva, J., Pallarés, F. J. and León Vizcaíno, L. 2005. Granulomatous hepatitis caused by *Salmonella Typhimurium* in a spur-thighed tortoise (*Testudo graeca*). *Vet. Rec.* **157**: 236–237. [Medline] [CrossRef]
4. Hervás, J., Sánchez-Cordón, P. J., de Chacón Lara, F., Carrasco, L. and Gómez-Villamandos, J. C. 2002. Hepatitis associated with herpes viral infection in the tortoise (*Testudo horsfieldii*). *J. Vet. Med. B Infect. Dis. Vet. Public Health* **49**: 111–114. [Medline] [CrossRef]
5. Huchzermeyer, F. W. and Cooper, J. E. 2000. Fibrinosis, not abscess, resulting from a localised inflammatory response to infection in reptiles and birds. *Vet. Rec.* **147**: 515–517. [Medline] [CrossRef]
6. Jacobson, E., Clubb, S. and Greiner, E. 1983. Amebiasis in red-footed tortoises. *J. Am. Vet. Med. Assoc.* **183**: 1192–1194. [Medline]
7. McArthur, S. 2006. Problem-solving approach to common disease of terrestrial and semi-aquatic chelonians. pp. 309–378. *In: Medicine and surgery of tortoises and turtles*, 2nd ed. (McArthur, S., Wilkinson, R. and Meyer, J. eds.), Blackwell Publishing Ltd., Oxford.
8. Ozaki, K., Matsuo, K., Tanaka, O. and Narama, I. 2000. Amoebiasis in the flat-shelled spider tortoise (*Acinixys planicauda*). *J. Comp. Pathol.* **123**: 299–301. [Medline] [CrossRef]
9. Raphael, B. L. 2003. Order Chelonia. pp. 48–58. *In: Zoo and Wild Animal Medicine*, 5th ed. (Fowler, E. M. and Miller, E. R. eds.), Saunders, St. Louis.
10. Rivera, S., Wellehan, J. F. X. Jr., McManamon, R., Innis, C. J., Garner, M. M., Raphael, B. L., Gregory, C. R., Latimer, K. S., Rodriguez, C. E., Diaz-Figueroa, O., Marlar, A. B., Nyaoke, A., Gates, A. E., Gilbert, K., Childress, A. L., Risatti, G. R. and Frasca, S. Jr. 2009. Systemic adenovirus infection in Sulawesi tortoises (*Indotestudo forsteni*) caused by a novel siadenovirus. *J. Vet. Diagn. Invest.* **21**: 415–426. [Medline] [CrossRef]
11. Wilkinson, R. 2006. Clinical pathology. pp. 141–162. *In: Medicine and surgery of tortoises and turtles*, 2nd ed. (McArthur, S., Wilkinson, R. and Meyer, J. eds.), Blackwell Publishing Ltd., Oxford.