was observed (data not shown). Thus, RAPD and mitochondrial DNA molecular data seem to indicate that the specimens here analyzed could belong to a sole taxon. In this case, the morphological features that support the specific differentiation of *N. emydis* and *N. emyditoides* (Fisher, 1960; Dezfuli et al., 1996) appear to be unreliable. The observed plasticity of morphological characters in co-occurring *Neoechinorhynchus* species parasites of North American turtles is not surprising because misclassification of females can frequently occur also among well differentiated species (from 9.5% to 34.3% of the sample; see Aho et al., 1992).

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Toxoplasma gondii Oocyst Survival under Defined Temperatures

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ABSTRACT: The survival of sporulated *Toxoplasma gondii* oocysts in water at −10 C to 70 C for various periods was investigated. Infectivity of *T. gondii* was tested by bioassay in mice. There was no marked loss of infectivity of oocysts stored at 10 C, 15 C, 20 C, and 25 C for 200 days, whereas there was a 100-fold loss of infectivity of oocysts stored at 30 C for 107 days. Oocysts stored at 35 C were infective for 32 days but not 62 days, at 40 C oocysts were infective for 9 days but not 28 days, at 45 C, oocysts were infective for 1 day but not 2 days, at 50 C oocysts were infective for 1 hr but not 2 hr. At 55 C and 60 C oocysts were rendered noninfective in 2 and 1 min, respectively. Oocysts re-

mained infective up to 54 mo at 4 C and there was no loss of infectivity in oocysts stored for 106 days at −5 C and at −10 C and for 13 mo at 0 C.

Infections by *Toxoplasma gondii* are widely prevalent in humans throughout the world. In general, *T. gondii* infections are more prevalent in mild temperature climates than in a hot and dry environment (reviewed in Dubey, 1979; Dubey and Beattie, 1988). Some of these epidemiological differences are related to the survival of oocysts in the environment. It is well known that *T. gondii* oocysts are resistant to environmental influences including drying and freezing (Dubey et al., 1970; Yilmaz and Hopkins, 1972; Frenkel and Dubey, 1972, 1973; Frenkel et al., 1975; Yamaura, 1976; Kutičič and Wikerhauser, 1994). However, survival of oocysts at different temperatures has not been critically investigated. The recent large-scale outbreak of acute toxoplasmosis linked to a water reservoir in British Columbia, Canada added another dimension to the epidemiology of oocyst-transmitted *T. gondii* infections (Bowie et al., 1997). The objective of the present study was to study survival of *T. gondii* oocysts at defined temperatures.

Oocysts of the VEG strain of *T. gondii* were obtained from feces of experimentally infected cats as described (Dubey et al., 1996). Oocysts were separated from cat feces, sporulated in 2% H_2SO_4 at room temperature for 1–4 wk, and then stored at 4 C. Before each experiment, H_2SO_4 was neutralized with 3.3% sodium hydroxide, the oocyst suspension was diluted 10-fold in 0.85% NaCl solution (saline), and aliquots of each dilution were bioassayed in mice. The number of oocysts in the inoculum refers to the number of mouse infective oocysts and not the visual counts. For this, oocysts were serially diluted 10-fold (10^{-1}–10^{-7}) and aliquots of each dilution were fed to mice. The last infective dilution was considered to have 1 infective oocyst (Dubey et al., 1997). The VEG strain of *T. gondii* was isolated in 1989 from blood of an acquired immune deficiency syndrome (AIDS) patient (Dubey et al., 1996). The VEG strain was selected for this study because it produces many oocysts in cat feces and tissue cysts in mouse brains. The same pool of oocysts was used in a given experiment. Mice inoculated orally with 1 oocyst of VEG strain become ill and 100 oocysts are often fatal to mice by the oral route (Dubey et al., 1997).

Swiss-Webster 20–25-g female mice (Taconic Farms, Germantown, New York) were used for bioassay. The day of death of inoculated mice was recorded to provide an idea of the relative number of viable oocysts in the inoculum because mortality is correlated with number of oocysts fed (Dubey and Frenkel, 1973; Dubey et al., 1997).

All inoculated mice were examined for *T. gondii* infection. Imprints of lungs and brain of the mice that died were examined for *T. gondii* tachyzoites or tissue cysts (Dubey and Beattie, 1988). Survivors were bled from the orbital sinus 2 mo after inoculation and a 1:25 dilution of serum was screened by the modified agglutination test (MAT) for *T. gondii* antibodies (Dubey and Desmonts, 1987). After serologic examination, all mice were killed and their brains were examined for tissue cysts. For this, a 2–3-mm portion of cerebrum from each mouse was squashed between a glass slide and a coverslip and examined microscopically for tissue cysts (Dubey and Beattie, 1988). Mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were demonstrated in murine tissues.

TABLE I. Survival of *T. gondii* oocysts at 35–45 C for various times.

Temperature (C)	Exposure time	Infectivity to mice: No. of mice infected with *T. gondii*/ no. of mice fed oocysts	Infectivity to mice: Day of death
	32 days	4/4	9–11
35	62 days	0/4	S*
	2 days	4/4	6–7
	9 days	4/4	9–10
	16 days	0/4	S
	21 days	1/4	S
40	28 days	0/4	S
	1, 2, 3, 6 hr	8/8†	6–7
	24 hr	2/2	S
45	3, 4, 7, 11 days	0/8†	S
50	30 min	2/2	13, S
	60 min	2/2	S
	120, 180 min	0/4†	S
55	10, 20, 30, 60 min	0/8†	S

*S = survived for 2 mo.
†Two mice were fed for each exposure time.

Mice were considered not infected with *T. gondii* when antibodies were not demonstrable in 1:25 dilution of serum obtained 2 mo postinoculation with test material; with the VEG strain, tissue cysts are found in all seropositive mice.

Two types of programmable temperature-controlled circulating baths with temperature stability of ±0.1 C were used. The temperatures were adjusted using water in a water bath (Precision Instruments, Chicago, Illinois; referred to in this paper as water bath type A) or by a mixture of ethylene glycol and water in another type of circulating bath (model 9101, Fisher Scientific, Pittsburgh, Pennsylvania; referred to as water bath B). The temperature was checked twice daily.

Five experiments were performed. Experiments 1 and 2 were performed using water bath type B, experiments 3 and 4 were performed in water bath type A, and experiment 5 was performed using a domestic refrigerator. In experiments 1–4, ~10^5 infective oocysts were used for each exposure.

In experiments 1–3, oocysts were dispensed in 0.5-ml screw-top polypropylene conical 4-cm-long vials (Saf-T-seal, USA Scientific, Ocala, Florida). The vials were inserted in Round Bubble Racks TPX (J&H Berge, Inc., Plainfield, New Jersey) that were floated in the water bath. In experiment 1, the water baths were set at 10 C, 15 C, 20 C, 25 C, and 30 C. After 10, 24, 29, 57, 94, and 200 days, 1 vial of oocysts was removed from the water bath and its contents were fed to 4 mice. In experiment 2, oocysts were stored at 0, −5, and at −10 C for 106 days. The temperature in the bath set at −10 C fluctuated between −6 and −10 C; therefore, the experiment was terminated at 106 days. In experiment 3, survival of oocysts at 35–55 C was investigated (see Table I).

In experiment 4, the effect of exposure at 50–70 C was studied by using heat-sealable plastic pouches so that oocysts were exposed instantly to the desired temperature. One milliliter of water containing ~10^5 infective oocysts was dispensed into 10

TABLE II. Survival of *T. gondii* oocysts at 52–70 C exposed for 1–5 min.

Trial no.	Temperature (C)	Exposure time (min)	Infectivity to mice: No. of mice positive for *T. gondii*/ no. of mice fed oocysts*	Infectivity to mice: Day of death
	65	1, 2, 5	0/6	S†
1	70	1, 2, 5	0/6	S
	60	1, 2, 5	0/6	S
2	55	1, 2, 5	0/6	S
	52	1	2/2	5–6
		2	2/2	7
		5	2/2	S
3	55	1	2/2	S
		2	0/2	S
4	55	1	0/2	S
	60	1	0/6	S
5	60	2	0/6	S

*For each treatment, 2 mice were fed oocysts.
†S = survived for 2 mo.

× 15-cm boilable plastic bags (Kapak/Scotchpak, Kapak Corporation, Minneapolis, Minnesota). Each bag was sealed 5 times to prevent oocyst spillage. After stated exposure time, pouches were removed immediately, submerged in ice-cold water for 1 min, and then bioassayed in mice. Five trials were performed (see Table II).

In experiment 5, the effect of storage of oocysts at 4 C was studied as an afterthought of another investigation. During the course of characterization of *T. gondii* isolates from hearts of naturally infected sows, oocysts were obtained from porcine isolates. Sporulated oocysts were initially stored in 2% aqueous H_2SO_4 and bioassayed in mice. For bioassay, the oocyst suspension was neutralized with 3.3% NaOH, and aliquots were fed to mice (Dubey et al., 1995). After testing for this paper (Dubey et al., 1995) was finished, the remaining inoculum had been stored in water at 4 C. After 1 interval of 34–54 mo, the oocysts from different samples were removed from the refrigerator, diluted with water, homogenized in a vortex to break up the bacterial and fungal growth, centrifuged, and fed to 2–4 mice to test for *T. gondii* infectivity.

In experiment 1, all mice fed oocysts stored at 10–25 C for as long as 200 days died of acute toxoplasmosis. Mouse bioassay of 10-fold dilutions of oocysts stored at 10–25 C for 200 days showed that there was no loss of infectivity of oocysts compared with control oocysts stored at 4 C. Mouse bioassay of oocysts stored at 30 C for 107 days indicated a 100-fold loss of infectivity.

In experiment 2, judging from the day of the death of mice, there appeared to be no loss of infectivity of oocysts stored for 106 days at −5 C and −10 C, and for 13 mo at 0 C.

In experiment 3, oocysts were rendered noninfective by storage for 62 days at 35 C, 28 days at 40 C, 2 days at 45 C, 120 min at 50 C, and 10 min at 55 C (Table I).

TABLE III. Infectivity of *T. gondii* oocysts stored at 4 C.

Months stored	Infectivity to mice
34	4/4*
35	4/4
38	7/8
39	7/7
40	8/9
42c	2/2
46	9/9
53	1/1
54	3/3

*No. of *T. gondii* isolates survived/no. of isolates bioassayed in mice.

In experiment 4, oocysts were rendered not infective by 1 min exposure at 60 C (Table II).

In experiment 5, 45 of 47 inocula of oocysts survived for 34 to 54 mo (Table III).

Results obtained in this investigation are in general agreement with published data and add to earlier findings concerning resistance of *T. gondii* to extremes in temperature (Dubey et al., 1970; Ito et al., 1975; Kutičič and Wikerhauser, 1994). Minor differences in results obtained earlier and those in the present investigation are probably due to different methods used to test temperature exposure. For example, in earlier experiments of Dubey et al. (1970) and Kutičič and Wikerhauser (1994), oocysts were killed by a 30-min exposure at 55 C and 15 min at 58 C in glass tubes in a conventional water bath without knowing the time needed to reach the desired temperature inside glass tubes. In the present study using plastic pouches with good thermal conductivity, it was determined that oocysts were killed within 2 min at 55 C and within 1 min at 60 C.

This investigation also provides data concerning survival of oocysts at 30–45 C, a temperature that is reached in several areas of the world. It is of interest that in moist conditions, oocysts survived for 32 days at 35 C, 9 days at 40 C, and only 1 day at 45 C. Yilmaz and Hopkins (1972) reported that *T. gondii* oocysts survived up to 306 days at 37 C, which is different than the results obtained in this investigation. Reasons for these divergent findings are not known.

Hutchison (1967) reported that oocysts in water survived at room temperature for 15 mo and Dubey (1977) reported survival of *T. gondii* oocysts for a similar period (548 days) in 2% H_2SO_4 at 4 C. Oocysts survived outdoors in feline feces for 46–548 days in Costa Rica and the United States depending on weather (Yilmaz and Hopkins, 1972; Frenkel et al., 1975). In the present study *T. gondii* oocysts survived up to 54 mo at 4 C in water without any preservative, but the data were qualitative. Although oocysts survive in water without preservatives, the growth of fungi makes counting of oocysts difficult and the effects of fungal and bacterial products on oocyst survival have not been investigated. In an earlier experiment, there was no loss of infectivity of oocysts stored at 4 C for 18 mo (Dubey et al., 1996). Therefore, oocysts should be stored in 2% H_2SO_4 and not longer than 18 mo to obtain reliable results.

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Leishmania-like Protozoan Associated with Dermatitis in Cattle

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ABSTRACT: A *Leishmania* sp.-like organism was found in the skin of a naturally infected 8-mo-old Red Dane female calf (*Bos taurus*) from Zimbabwe. There were multiple alopecic nodules, particularly on the face and udder. The nodules were up to 5 cm in diameter and larger ones were ulcerated and hemorrhagic. Numerous *Leishmania*-like amastigotes were seen in the skin lesions. Ultrastructurally, the organisms were oval to elongated (2–2.5 μm long), had a nucleus, a kinetoplast, and a flagellum. To our knowledge, this is the first report of *Leishmania*-like organisms found in an animal from Zimbabwe.

Species of the genus *Leishmania* are parasites of humans, carnivores, rodents, equids, and some wild animals in many countries (Levine, 1973; Van der Lugt et al., 1992; Bowman, 1995). Van der Lugt et al. (1992) reported the first case of dermal leishmaniasis in an adult sheep from Eastern Transval, South Africa. We report leishmaniasis in a calf from Zimbabwe.

An 8-mo-old Red Dane female calf (*Bos taurus*) was submitted for necropsy to the Department of Paraclinical Veterinary Studies, University of Zimbabwe, Harare, Zimbabwe. The animal had developed skin lesions consisting of multifocal ulcerative nodules throughout the body 2 mo before necropsy. No other details were available concerning the case history or history of other animals in this herd.

Gross lesions were limited to the skin and were characterized by multiple alopecic nodules, particularly on the face and udder. The nodules were 1–5 cm in diameter, up to 4 cm thick, and the larger ones were ulcerated and bleeding.

Specimens of skin, lung, liver, spleen, kidney, ureter, adrenal, bone marrow, and subileac lymph nodes were fixed in 10% buffered neutral formalin. Paraffin embedded tissues were processed routinely and 5–6-μm-thick sections were examined after staining with hematoxylin and eosin (HE), Giemsa's stain, periodic acid Schiff (PAS) reaction, Ziehl–Nielsen stain, and Gamori's methanemine silver (GMS). Retrospectively, a portion of paraffin-embedded skin was deparaffinized, postfixed in osmium, and processed for transmission electron microscopy (TEM).

Important microscopic lesions were confined to the skin and consisted of extensive ulceration with serocellular exudate and necrotic debris (Fig. 1A). There was an extensive mixed-type dermal inflammatory cell infiltration, including large numbers of neutrophils, macrophages, plasma cells, and few lymphocytes (Figs. 1, 2). The dermis was thickened 2–3 times normal by increased fibrous connective tissue and enmeshed fibroblasts (Fig. 1B). The subileac lymph node had massive lymphoid hyperplasia with expansion of follicles.

There were numerous protozoa present that were barely visible in 5–6-μm-thick HE-stained sections. Examination of 1–3-