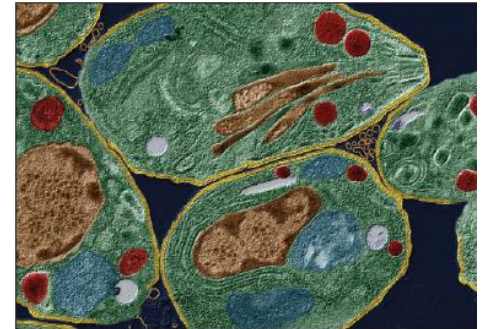


Solid organ nakli alıcılarında toksoplazmoz

Dr. Özlem Kurt Azap
KLİMİK Ankara Toplantıları
26 Aralık 2012



DR. ERSİN ARSLAN



**UNUTMAYACAĞIZ
UNUTTURMAYACAĞIZ**



Dr. Melike Erdem



Dr. Mustafa Bilgiç

ARTIK YETER!



Türkiye’de Organ Nakli

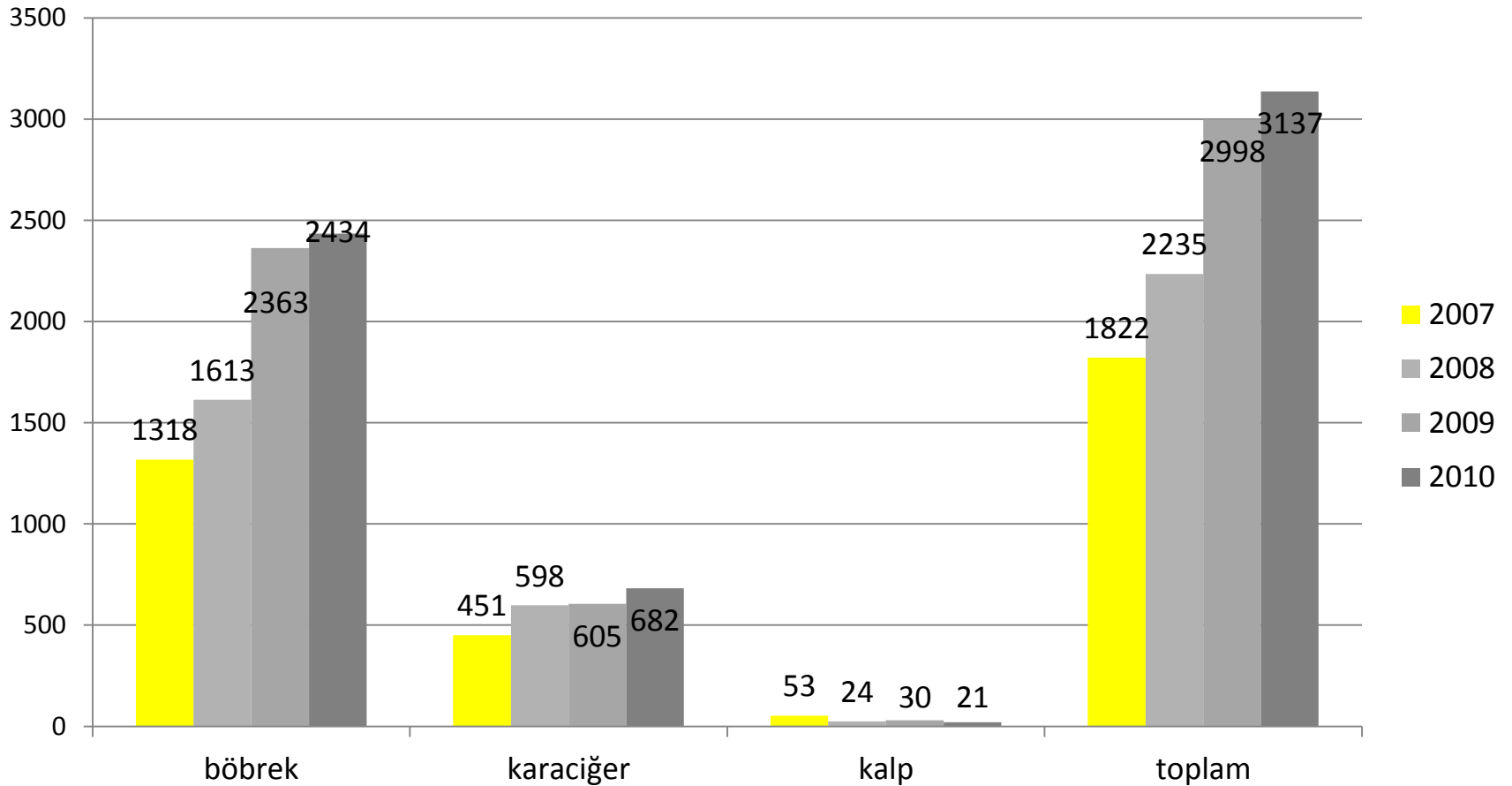
1975-2004

Transplantation Activities in Turkey From November 1975 - January 2004

Organ/Tissue Donor	Cadaveric Donor	Living Donor	Total
Kidney	1624 (24.3%)	5062 (75.7%)	6686
Liver	433 (62.3%)	263 (35.7%)	696
Heart	132	0	132
Heart valve	185	0	185
Pancreas	15	0	15
Cornea	13278	0	13278
Bone marrow	0	2883	2883

2007-2010

Organ transplantasyon sayıları



Hastalık etkenleri

- İnsanlarda hastalık etkeni olan tür sayısı: 1407
- Bunların %58'i zoonotik
- 177'si “emerging veya re-emerging”
- Zoonotik olanların sayısı non-zoonotiklerden 2 kat fazla



Zoonoses in Solid-Organ and Hematopoietic Stem Cell Transplant Recipients

Camille N. Kotton

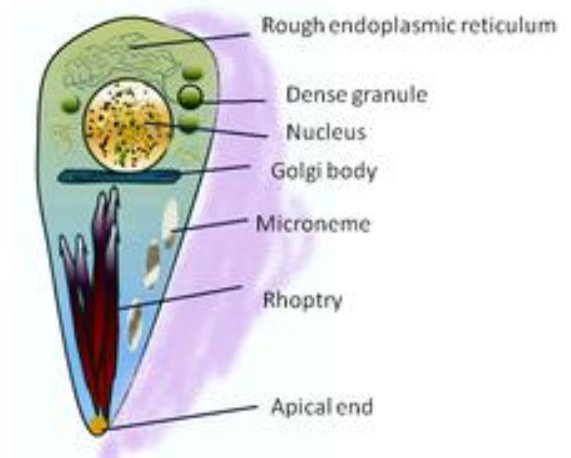
Transplant and Immunocompromised Host Section, Infectious Diseases Division, Massachusetts General Hospital, Boston, Massachusetts

Parasite

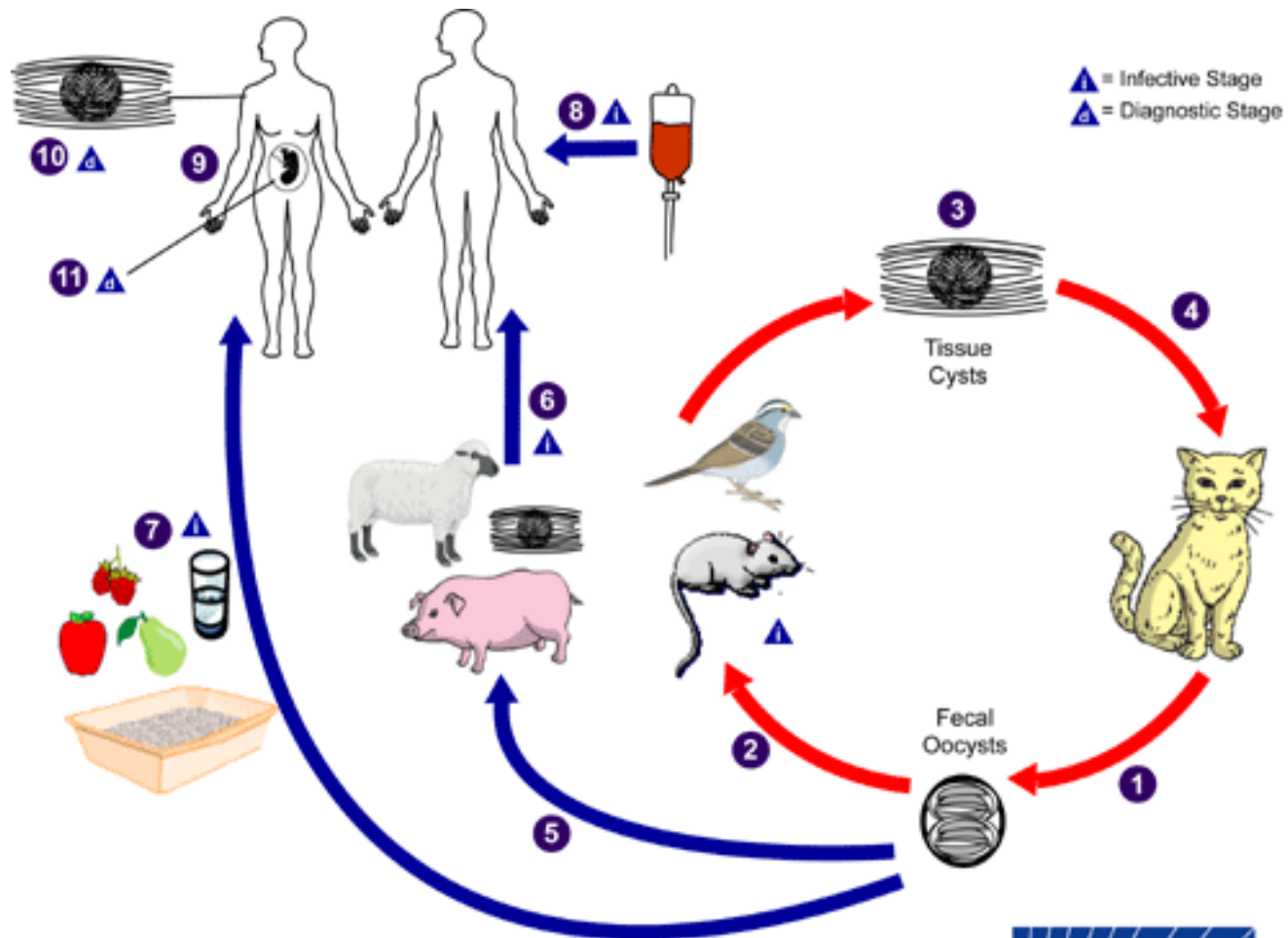
<i>Babesia</i> species	Tick bite	Heart [85] and kidney [86–88]	No	Rodents, cattle
<i>Clonorchis sinensis</i>	Ingestion	BM (before transplantation) [89] and liver [90–92]	No	Fish
<i>Cryptosporidium parvum</i>	Ingestion	Liver [93, 94], kidney [95, 96], and HSC [97]	No	Numerous
<i>Echinococcus granulosus</i>	Ingestion	Liver [98–101] and heart [102]	No	Dogs, sheep
<i>Giardia lamblia</i>	Ingestion	Intestine [103] and HSC [104]	No	Numerous
<i>Leishmania</i> species	Insect bite	Numerous SOs [105–115] and HSC [116]	No	Rodents, dogs
Microsporidia	Ingestion	Kidney [117], kidney-pancreas [117], and BM [118, 119]	No	Numerous
<i>Taenia solium</i>	Ingestion	Kidney [120, 121]	No	Swine
<i>Toxoplasma gondii</i>	Ingestion	Numerous	Yes [122, 123]	Cats, cattle, goats, sheep
<i>Trypanosoma cruzi</i>	Insect bite	Kidney [124], liver [124, 125], and pancreas [124]	Yes [124, 125]	Rodents, wildlife, house pets

Toksoplazma gondii

- 1908- kemirgenlerde gösterildi
- 1923- konjenital hastalık
- 1968- İmmünekompromize kişilerde hastalık

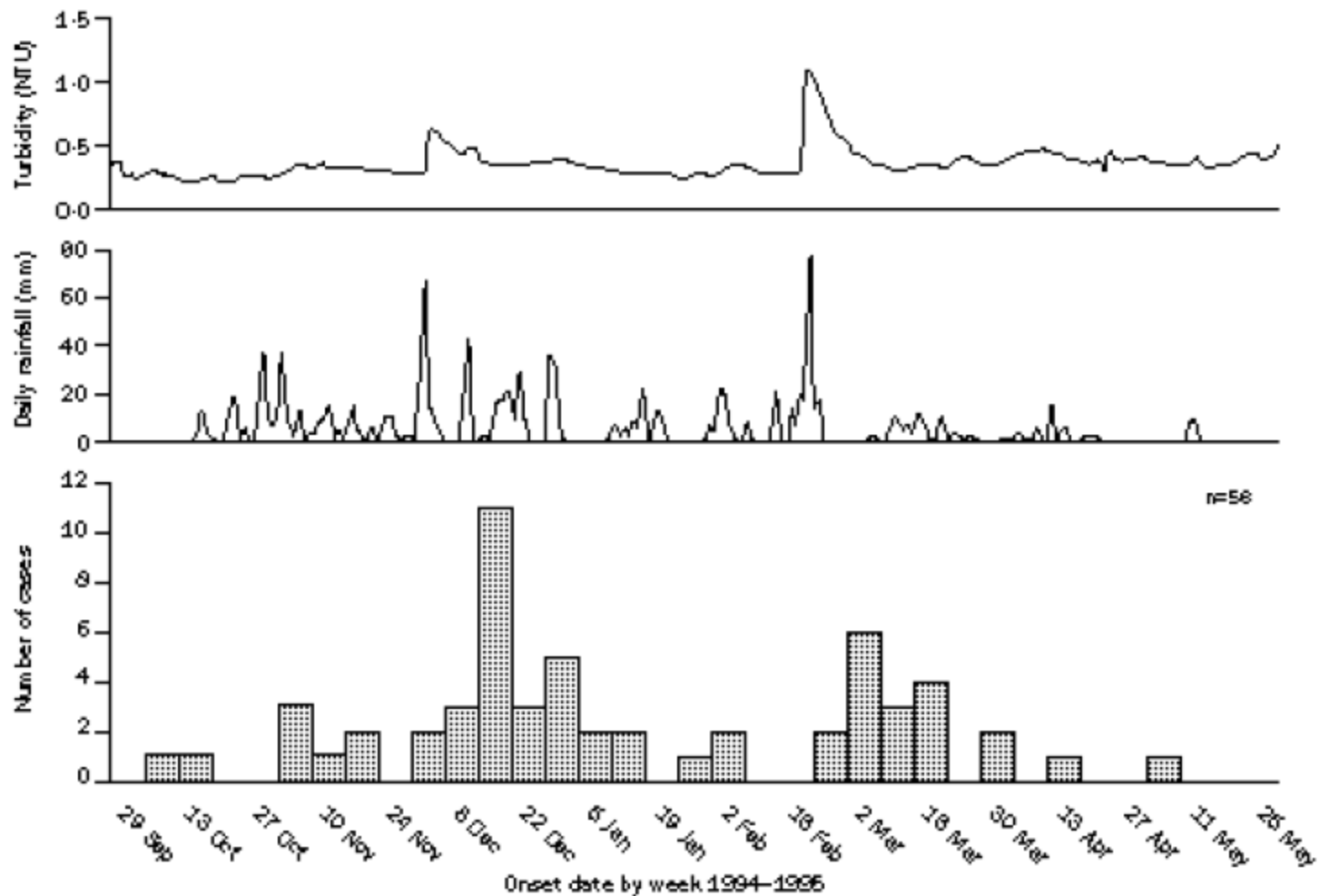


Bulaş yolları



İçme suyu!

Outbreak of toxoplasmosis associated with municipal drinking water

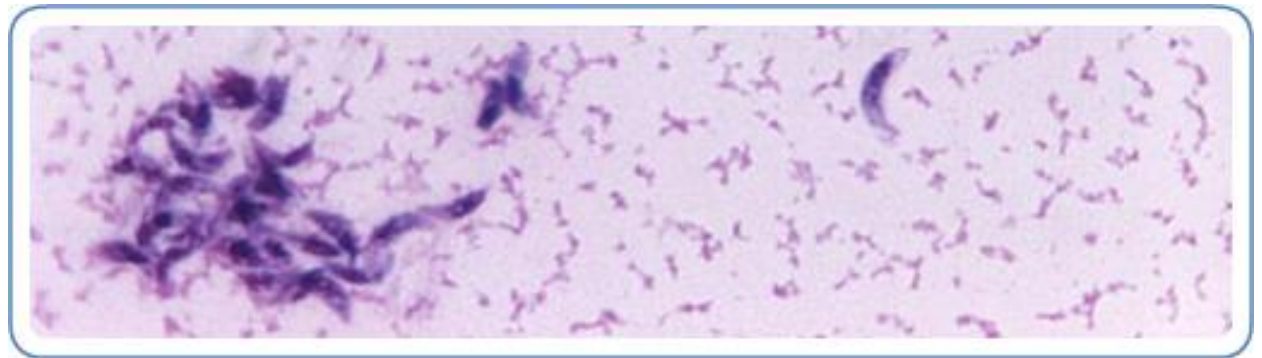


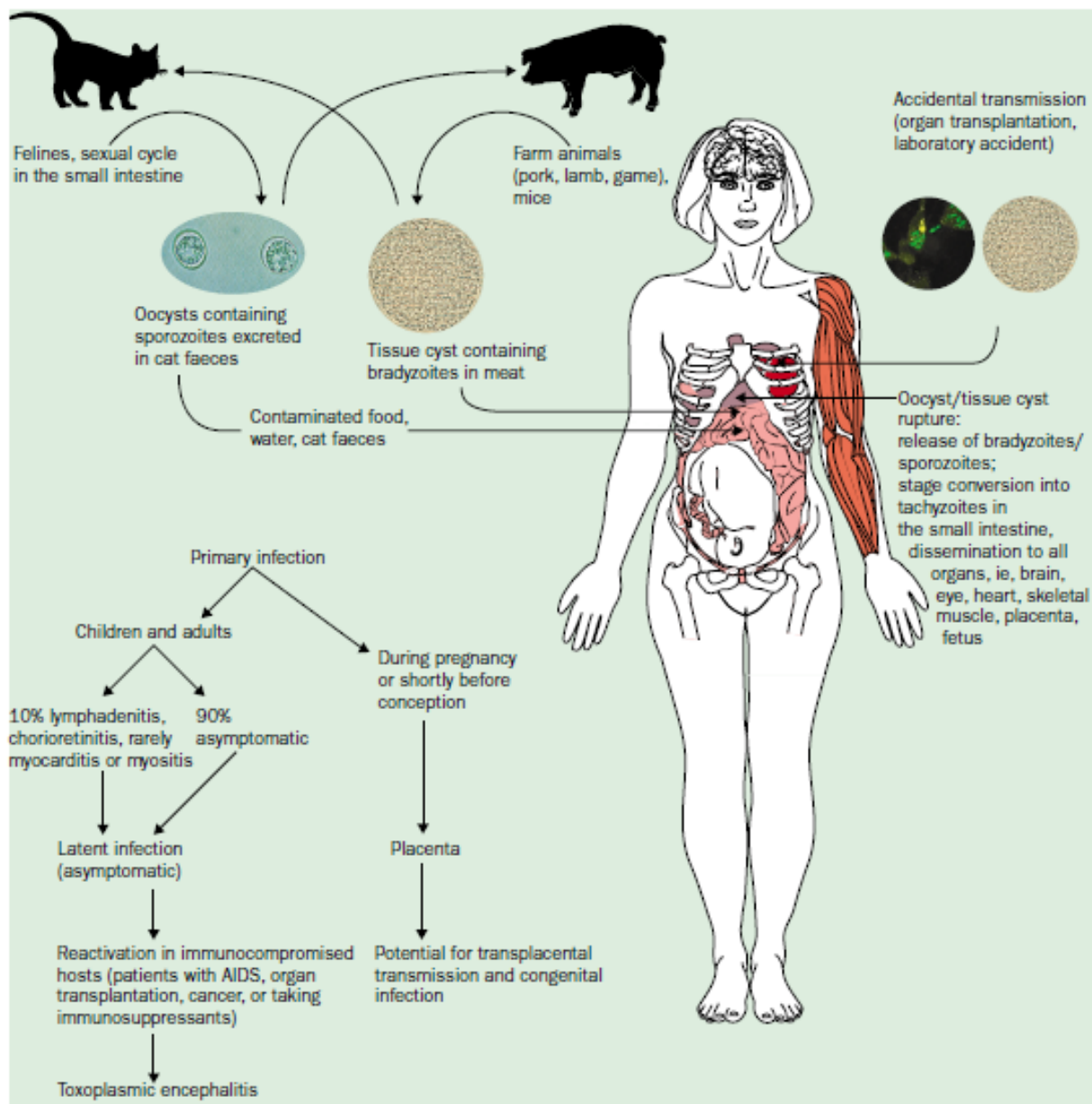
“İhmal edilmiş” 5 paraziter enfeksiyon- CDC

Neglected Parasitic Infections in the United States

The Five Targeted Infections

CDC has targeted five parasitic infections as priorities for public health action, based on the numbers of people infected, the severity of the illnesses, or our ability to prevent and treat them. These include Chagas disease, neurocysticercosis, toxocariasis, toxoplasmosis, and trichomoniasis.





Kayseri Kapalı Cezaevi Mahkumlarında *Toxoplasma gondii* Seroprevalansı

yabani hayvanlarda görülebilen bir zoonozdur. Bu çalışmada, Kayseri Kapalı Cezaevi'nde bulunan 628 mahkumda toksoplazmozis seroprevalansının araştırılmasını amaçlanmıştır. Alınan kan örneklerinde anti-*Toxoplasma gondii* IgG ve IgM antikorları IFA yöntemi ile araştırılmıştır. Mahkumların 236 (%37,58)'sında anti-*T.gondii* IgG seropozitifliği, 11 (%1,75)'inde ise IgG ve IgM'nin bir arada seropozitifliği saptanmış, IgG negatif olan serum örneklerinin hiçbirinde IgM antikorları pozitif olarak bulunmamıştır

Tablo 2. Yaşa göre anti-*T.gondii* IgG ve IgM antikorlarının dağılımları

Yaş grupları	Toplam Kişi		Anti- <i>T.gondii</i> IgG				Anti- <i>T.gondii</i> IgM			
			Negatif		Pozitif		Negatif		Pozitif	
	n	%	n	%	n	%	n	%	n	%
0-19	45	7,2	29	64,4	16	35,6	45	100	-	-
20-44	512	81,5	316	61,7	196	38,3	502	98,0	10	2,0
45-60	55	8,8	37	67,3	18	32,7	54	98,2	1	1,8
61-74	11	1,8	7	63,6	4	36,4	11	100	-	-
75 ve üzeri	5	0,8	3	60,0	2	40,0	5	100	-	-
Toplam	628	100	392	62,43	236	37,58	617	98,25	11	1,75

χ^2 : 1,210, p>0.05

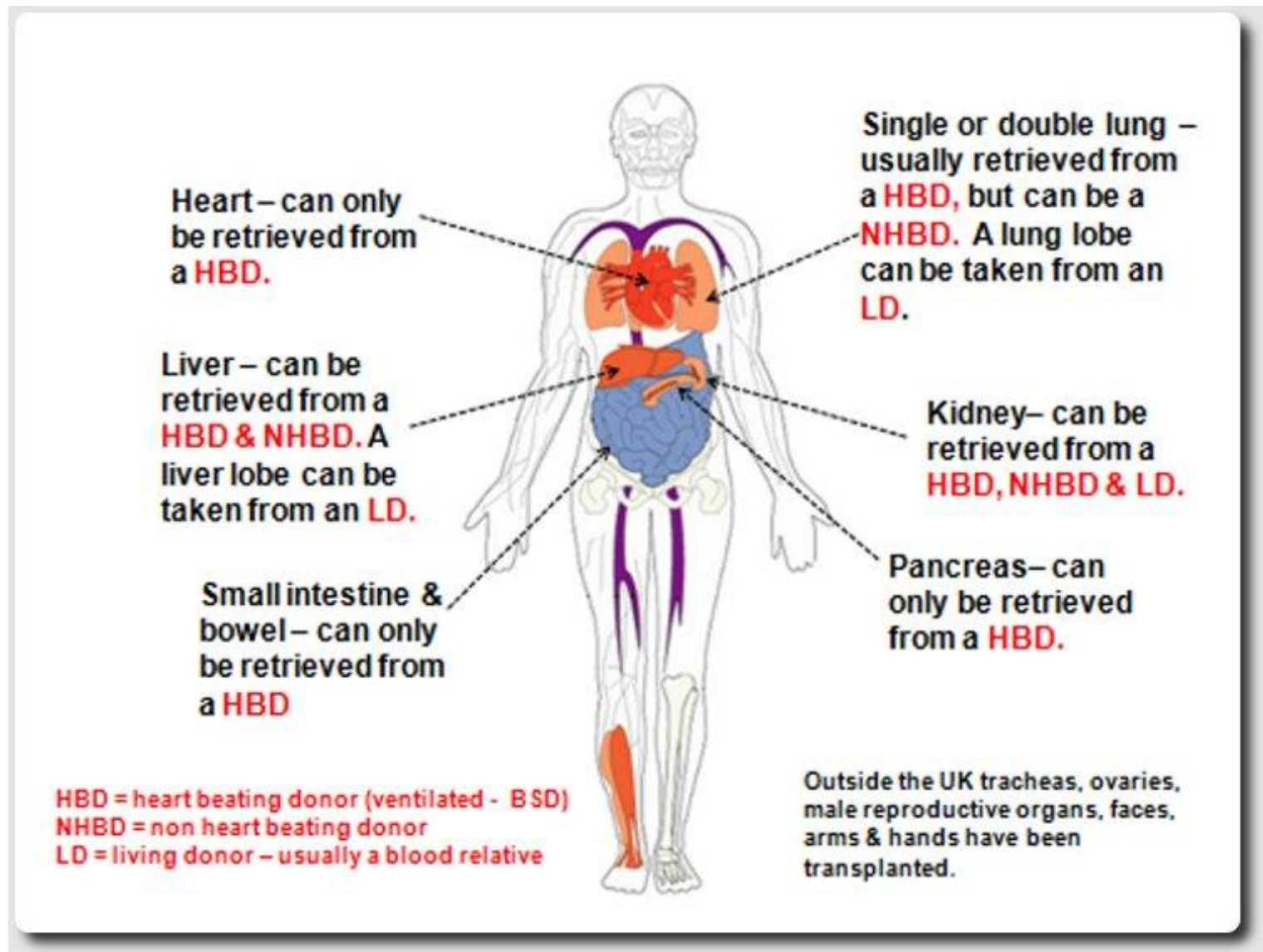
Fertil ve İnfertil Kadınlarda *Toxoplasma gondii* Seropozitifliğinin Retrospektif Olarak Değerlendirilmesi

riier retrospektif olarak değeriendirinimiştir. Çalışmaya, 2004-2009 yılları arasında ülkemizin değişik bölgelerinden Ankara'da bulunan özel bir IVF merkezine, primer ve sekonder infertilite nedeniyle başvuran çiftler arasından 1314 kadın olgu (ortalama yaş: 31.8 ± 5.6 yıl) alınmıştır. Çalışma grubu bütün olarak değerlendirildiğinde; 376 (%28.6) kadında IgG, 5 (%0.4)'inde IgG + IgM ve 1 (%0.07)'inde sadece IgM olmak üzere toplam 382 (%29.1)'sinde toksoplazma seropozitifliği belirlenmiştir. Değerlendirilen çiftler.

Gebelerde Toksoplasma gondii Seroprevalansı

açabilir. Ocak 2007- Aralık 2008 tarihleri arasında normal gebelik takibi amacıyla Adıyaman 82. Yıl Devlet Hastanesi Kadın Doğum Poliklinikleri'ne başvuran 17-45 yaş arasındaki 455 gebede Toxoplasma gondii seroprevalansı retrospektif olarak araştırıldı. Anti-Toxoplasma gondii IgG seropozitiflik oranı % 48,4 (220/455) ve IgM pozitiflik oranı ise % 0,65 (3/455) olarak saptandı. Sonuç

“Donor transplant education group” - İngiltere



Parasitic Infections in Solid Organ Transplant Recipients

C. N. Kotton^{a,*}, R. Lattes^b and the AST Infectious Diseases Community of Practice

Recommendations

- (1) All pre-heart transplant recipients and donors should be serotested for *Toxoplasma* (Grade II). It is not clear that other organ transplant recipients and donors need to be tested (7).
- (2) After heart transplant, prophylaxis should be given, as outlined earlier. Disease has been seen after cessation of prophylaxis, the optimal duration of prophylaxis has not been determined, and it is given for life at many transplant centers (Grade III).
- (3) Acute toxoplasmosis can have protean manifestations and should be included in the differential diagnosis of infectious syndromes after organ transplant.
- (4) Treatment of acute toxoplasmosis is not well-studied in solid organ transplant recipients; much of our knowledge comes from the treatment of HIV+ patients.

Table 2: Quality of evidence on which a recommendation is based

Grade	Definition
I	Randomized controlled trials
II-1	Controlled trials without randomization
II-2	Cohort or case-control analytic studies
II-3	Multiple time series, dramatic uncontrolled experiments
III	Opinions of respected authorities, descriptive epidemiology

Risk Factors, Clinical Features, and Outcomes of Toxoplasmosis in Solid-Organ Transplant Recipients: A Matched Case-Control Study

Table 1. Univariate Analysis of Risk Factors for Toxoplasmosis in Solid-Organ Transplant Recipients

Variable	Case Patients (n = 22)		Control Subjects (n = 44)		OR	(95% CI)	P Value
Male sex	16	(72.7)	35	(79.5)	1.45	(.44–4.79)	.547
Age, years, median (range)	50.5	(20–68)	53.9	(18–67)	0.98	(.94–1.03)	.476
D/R Toxoplasma serostatus ^a							
D+/R–	9	(40.9)	2	(4.5)			<.001
D unknown/R–	8	(36.4)	3	(6.8)			
D–/R–	1	(4.5)	6	(13.6)			
D–/R+	...		1	(2.3)			
D+/R+	1	(4.5)	9	(20.5)			
D unknown/R+	1	(4.5)	9	(20.5)			
D+/R unknown	1	(4.5)	2	(4.5)			
D unknown/R unknown	1	(4.5)	12	(27.3)			
Negative serostatus before transplantation ^a	18	(90.0)	11	(36.7)	15.54	(3.02–80.04)	<.001
Diabetes mellitus	3	(13.6)	7	(15.9)	0.83	(.19–3.59)	1.000
Previous blood transfusions	3	(13.6)	4	(9.1)	1.58	(.32–7.77)	.429
Prophylaxis with TMP-SMZ at any time since transplantation	8	(36.4)	20	(45.5)	0.69	(.24–1.96)	.481
Prophylaxis with pyrimethamine at any time since transplantation	2	(9.1)	...		0.31	(.217–.45)	.108

Table 3. Clinical Characteristics, Treatment, and Outcomes of 22 Solid-Organ Transplant Recipients With Toxoplasmosis

Variable	Patients, No. (%)	
Male sex	16	(72.7)
Age, years, median (range)	50.02	(20–68)
Transplant		
Heart	12	(54.5)
Kidney	6	(27.2)
Liver	4	(18.2)
Primary toxoplasmosis ^a	18	(81.8)
Days to symptom onset after transplantation, median (range)	86.5	(12–7097)
Days of symptoms until diagnosis, median (range)	12	(0–53)
Clinical manifestations		
Temperature >38°C	14	(63.6)
Dyspnea	7	(31.8)
Cough	6	(27.3)
Headache	6	(27.3)
Confusion	6	(27.3)
Focal neurologic signs	5	(22.7)
Visual abnormalities	3	(13.6)
Hepatosplenomegaly	2	(9.1)
Lymph node enlargement	2	(9.1)
Shock at presentation	2	(9.1)
Site of <i>Toxoplasma</i> infection		
Pneumonitis	7	(31.8)
Myocarditis	5	(22.7)
Brain abscesses	5	(22.7)
Chorioretinitis	3	13.6
Meningitis	1	(4.5)
Disseminated disease	5	(22.7)

Variable	Patients, No. (%)	
Days of hospitalization, median (range) ^b	23	(0–120)
Crude mortality	3	(13.6)

Infectious Complications among 620 Consecutive Heart Transplant Patients at Stanford University Medical Center

Jose G. Montoya,^{1,2} Luis F. Giraldo,^{1,2} Bradley Efron,⁵ Edward B. Stinson,⁴ Pat Gamberg,⁴ Sharon Hunt,³ Nadia Giannetti,³ Joan Miller,⁴ and Jack S. Remington^{1,2}

¹Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, Palo Alto; and ²Division of Infectious Diseases and Geographic Medicine and ³Division of Cardiovascular Medicine (Department of Medicine), ⁴Department of Cardiothoracic Surgery, and ⁵Department of Statistics and Biostatistics, Stanford University School of Medicine, Stanford, California

A total of 1073 infectious episodes (IEs) that occurred in 620 consecutive heart transplantation patients at Stanford Medical Center between 16 December 1980 and 30 June 1996 were reviewed. Infectious complications were a major cause of morbidity and mortality, second only to rejection as the cause of early deaths and the most common cause of late deaths. Of the IEs, 468 (43.6%) were caused by bacteria, 447 (41.7%) by viruses, 109 (10.2%) by fungi, 43 (4.0%) by *Pneumocystis carinii*, and 6 (0.6%) by protozoa. The largest number of IEs occurred in the lungs (301 [28.1%]). A significant reduction in the incidence of IEs and a delay in presentation after transplantation were observed; these were most likely related to the introduction of new chemoprophylactic regimens during the study period and prevention of significant disease caused by cytomegalovirus.

The most common IE due to parasites was caused by *T. gondii* (4 of 6); the other 2 IEs were due to intestinal giardiasis and vaginal trichomoniasis. Results of serological testing for *Toxoplasma* were available for 582 donors (35 [6%] had *T. gondii*-specific IgG antibodies) and 607 recipients (98 [16.1%] were positive). Results of serological testing for *Toxoplasma* were available for 575 D/R pairs; of these, 454 (79%) were D⁻/R⁻, 84 (14.6%) D⁻/R⁺, 32 (5.6%) D⁺/R⁻, and 5 (0.8%) D⁺/R⁺. Of the 32 D⁺/R⁻ patients, 16 were receiving trimethoprim-sulfamethoxazole and/or pyrimethamine prophylaxis, and none of those 16 developed toxoplasmosis; however, 4 (25%) of the 16 D⁺/R⁻ patients who were not taking either trimethoprim-sulfamethoxazole or pyrimethamine developed toxoplasmosis, and all died of the infection. None of the 98 patients who were seropositive for *T. gondii* preoperatively developed clinical evidence of reactivation of the infection.

Tokso IgG pozitifliği
582 donörde %6
607 alıcıda %16

D⁻/R⁻ %79

D⁻/R⁺ %14.6

D⁺/R⁻ %5.6

D⁺/R⁺ %0.8

BAŞKENT DENEYİMİ- MESOT 2012

METHODS

- Retrospective study
- 66 cardiac transplant recipients (2003-2012)
- Pre and post transplant toxoplasma serology (Anti-Toxoplasma IgG) of the recipients and their donors were evaluated from laboratory records
- The outcome of mismatched recipients were also evaluated from medical records.

RESULTS

- Age: (1-61 years)
 - 27/66 (41%) <18 years
- Sex: 46/66 male
- Before transplantation;
 - Donor seroprevalence 26 %
 - Recipient seroprevalence 31 %

RESULTS

- D+/R- status 7/66 (mismatched)
 - D- /R- status 39
 - R + 20
- All patients planned to receive TMP/SMX prophylaxis
 - R+: 6 months
 - D-/R-: 1 year
 - D+/R-: life long
- Incidence of toxoplasmosis 3%

D+ /R-	Age	Sex	Compliance to TMP-SMZ prophylaxis	Status after Tx.	Toxo manifestation	Time to diagnosis After tx.	Prog nosis
TK	4	M	No	R+	Myocarditis	3 months	exitus
SH	13	F	Yes	R-	-	-	
HMA	13	F	Yes	R-	-	-	
İY	15	M	No	R+	Pneumonitis	21months	
SB	41	F	Yes	R-	-	-	
İB	54	M	Yes	R-	-	-	
KY	54	M	Yes	R-			exitus

***Toxoplasma gondii* primary infection in renal transplant recipients. Two case reports and literature review**

İki olgu: IgG'leri negatif iken TMP-SMZ kesildikten sonra klinik bulgular ile birlikte Ig M'leri pozitifleşmiş

Literatür araştırması(1966-2009): 20 primer toksoplazmoz

- 20 olgunun 10'unun eksitus
- Eksitus olanların tamamı post trans ilk 3 ay içinde
- %85'inde ateş

Donor-transmitted toxoplasmosis in liver transplant recipients: a case report and literature review

KC nakli sonrası 1. ayda solunum yetmezliği
BAL da ve serumda PCR pozitifliği

Reported cases of toxoplasmosis in liver transplant recipients

Year, reference	Interval	Clinical	Diagnosis	Pretransplant	Post-transplant	Medications	Outcome
1972, (12)	35 days	Fever Pneumonia	Autopsy			AZA, P	Died
1987, (13)	24 days	Fever Meningitis	Cell culture	D (IgG +) R (IgG +)	IgG rise	CSA, P, OKT3 AR	Survived
1993, (14)	9 months	Retinitis choroiditis	Microscopy		IgG + IgM –	AZA, CSA	Survived
1995, (5)	24 days	Fever Pneumonia	Autopsy	D (IgG +, IgM +) R (IgG–, IgM –)		ATG, AZA, P, CSA, OKT3 AR	Died
1996, (15)	27 days	Hypotension Pneumonia	BAL cell culture Autopsy	R (IgG +)		FK, MMF CR	Died
1996, (6)	50 days	Retinitis	PCR (anterior chamber fluid)	D (IgG +) R (IgG –)	IgM +		Survived
1998, (16)	125 days	Fever Sepsis Re-transplant	BAL microscopy Autopsy PCR (blood, liver)	D1/D2 (IgG –) R (IgG +)	IgG rise IgM –	MMF, AZA, CSA AR/CR	Died
2002, (17)	13 days	Seizure White matter lesions			IFT +	CSA, AZA, P, ATG	Survived
	88 days	Encephalitis			IFT +	CSA, AZA, P, ATG	Died
2002, (18)	22 days	Fever Pneumonia	BAL microscopy Autopsy	R (IgG +)	No IgG rise IgM –	P, FK, ATG	Died
2002, (19)	41 days	Fever Pneumonia	BAL microscopy PCR (BAL)	R (IgG +)	IgG rise IgM –	FK, P, Atgam AR	Survived
2002, (7)	12 days	Fever Pneumonia	BAL microscopy PCR (blood, BAL)	D (IgM –, IgG +) R (IgM –, IgG –)	IgG weak + IgM –	FK, MMF, P	Died

First Case of Toxoplasmosis Following Small Bowel Transplantation and Systematic Review of Tissue-Invasive Toxoplasmosis Following Noncardiac Solid Organ Transplantation

10 Y, incebarsak nakli yapılan hasta
Post trans 3. ayda ishal, karın ağrısı,
Solunum yetmezliği...eksitus
Postmortem incelemede
“yaygın tokosplazma kistleri”

1966-2005 literatür taraması:

- 52 kalp dışı solid organ nakli olgusu
- Primer enfeksiyon %42
- Reaktivasyon/reenfeksiyon %21
- Bilinmeyen %37

TABLE 3. Clinical features of reported cases of toxoplasmosis

Characteristic	n (%)
Median time from transplant to symptom onset, days (range)	27 (0–2555)
Onset (days post transplantation)	49
Days 0–30	32 (65)
Days 31–90	10 (20)
Days > 90	7 (14)
Mean time from transplant to symptom onset, days (range) ^a	
Standard risk	31 (15–2190)
High risk	16 (9–60)
Unknown risk	28 (0–2555)
Mortality	32 (65)
Presenting manifestations	50 (96)
Fever	40 (77)
Respiratory manifestations ^b	15 (29)
Neurologic manifestations ^c	13 (26)
Bone marrow suppression	13 (26)
Ophthalmologic manifestations ^d	5 (10)
Hepatitis	4 (8)
Splenomegaly	3 (6)
Graft failure	3 (6)
Diarrhea	3 (6)
Headache	3 (6)
Hepatomegaly	2 (4)
Disseminated disease	44 (85)
Fever	39 (91)
Pneumonitis	34 (77)
Bone marrow suppression	27 (63)
Central nervous system	24 (56)
Hepatitis	11 (26)
Carditis	10 (23)
Renal failure	4 (9)
Sepsis/shock	4 (9)
Localized disease	7 (13)
Ophthalmologic	4 (57)
Central nervous system	3 (43)
Isolated fever	1 (2)

TANI

	Antibody class/ test	Screening	Pregnancy	Newborns	Eye disease	Immunocompromised patients
Indirect detection/ serology	IgG	+	+ (identification of women at risk and those protected)	+ (maternal antibodies may persist until 12 months of age; differentiation of maternal and fetal IgG by western blot or ELISA)	+ (low titres are usually seen in patients with reactivation of congenital disease; intraocular antibody production [ratio of ocular and blood antibody titres])	+ (identification of patients at risk of reactivation, ie, AIDS, bone marrow transplant patients)
	IgG avidity	–	+ (high avidity results rule out infection in recent 3–4 months; low avidity antibodies may persist)	–	+ (high avidity results rule out infection in recent 3–4 months; low avidity antibodies may persist)	–
	IgM*	–†	+ (IgM antibodies may persist for prolonged times, negative IgM rules out infection in pregnant women during the first two trimesters)	+ (ISAGA more sensitive than EIA; differentiation of maternal and fetal IgG by western blot)	+ (high titres usually in patients with acute acquired disease, negative results in patients with reactivation of congenital disease)	+ (IgM of little value; may or not be present with active or latent disease)
	IgA	–	+ (IgA antibodies may persist for prolonged times)	+ (increased value compared to IgM tests)	–	–
	IgE	–	+ (high specificity, low sensitivity)	–	–	–
Direct detection	PCR	–	+ (amniotic fluid)	+ (blood, urine)	+ (particularly useful in patients with atypical retinal lesions or suboptimum response to therapy [vitreal or aqueous fluid, vitreal fluid preferred])	+ (cerebrospinal fluid, bronchoalveolar lavage, ocular fluids, ascitic fluid, pleural fluid, peritoneal fluid, bone marrow aspirate, peripheral blood, and/or tissue)
	Histology (immunohistochemistry†)/cell culture or mouse inoculation	–	+ (placenta and fetal tissues in cases of fetal loss)	–	–	+ (any affected tissue)
Comments/aims		Determination of sero-prevalence/epidemiological studies	Combined detection of IgG and IgM antibodies for screening in early pregnancy	Increased sensitivity of combined IgA and IgM antibody detection	Serological distinction between congenital and recently acquired infection	Direct detection more sensitive than indirect detection

TANI

TABLE 4. Diagnosis of toxoplasmosis

Test or procedure	N (%)
Autopsy alone	21 (40)
Serology	23 (44)
Direct histopathologic examination	10 (19) ^a
Bronchoalveolar lavage fluid	5
Bone marrow aspirate	3
Brain	1
Retina	1
Polymerase chain reaction	7 (13) ^a
Blood	4
Bronchoalveolar lavage fluid	3
Bone marrow	2
Aqueous humor	2
Cerebrospinal fluid	1
Culture	3 (6)
Blood	2
Bronchoalveolar lavage fluid	1
Mouse inoculation and direct visualization	3 (6) ^a
Bone marrow aspirate	3
Blood	2
Cerebrospinal fluid	1
Bronchoalveolar lavage	1

TEDAVi

Acute/primary treatment of toxoplasma encephalitis in patients with AIDS

Standard regimens

Pyrimethamine Oral 200 mg loading dose, then 50–75 mg qd

Leucovorin Oral, intravenous, or intramuscular
10–20 mg qd (up to 50 mg qd)

plus

Sulfadiazine Oral 1–1.5 g q6h

or

Clindamycin Oral or intravenous 600 mg q6h
(up to intravenous 1200 mg q6h)

At least 4–6 weeks after resolution of signs and symptoms

During and for 1 week after pyrimethamine treatment

**

**

Possible alternative regimens

Trimethoprim Oral or intravenous 5 mg (trimethoprim
sulfamethoxazole component)/kg q12h
(daily doses as high as 15–20 mg/kg of the
trimethoprim component have been used)

**

Pyrimethamine As in standard regimens

**

plus leucovorin

*plus one of the
following*

Clarithromycin Oral 1g q12h

**

Atovaquone Oral 750 mg q6h

**

Azithromycin Oral 1200–1500 mg qd

**

Dapsone Oral 100 mg qd

**

Parasitic Infections in Solid Organ Transplant Recipients

C. N. Kotton^{a,*}, R. Lattes^b and the AST Infectious Diseases Community of Practice

Recommendations

- (1) All pre-heart transplant recipients and donors should be serotested for *Toxoplasma* (Grade II). It is not clear that other organ transplant recipients and donors need to be tested (7).
- (2) After heart transplant, prophylaxis should be given, as outlined earlier. Disease has been seen after cessation of prophylaxis, the optimal duration of prophylaxis has not been determined, and it is given for life at many transplant centers (Grade III).
- (3) Acute toxoplasmosis can have protean manifestations and should be included in the differential diagnosis of infectious syndromes after organ transplant.
- (4) Treatment of acute toxoplasmosis is not well-studied in solid organ transplant recipients; much of our knowledge comes from the treatment of HIV+ patients.

Table 2: Quality of evidence on which a recommendation is based

Grade	Definition
I	Randomized controlled trials
II-1	Controlled trials without randomization
II-2	Cohort or case-control analytic studies
II-3	Multiple time series, dramatic uncontrolled experiments
III	Opinions of respected authorities, descriptive epidemiology

Nakil hastaları &



- Topraklarını mümkünse başkası deęiřtirsın
- Eldiven kullanımı ve el yıkama önemli
- Kedi dıřarı gitmemeli



Nakil hastaları &



- Etler 66°C'nin üzerine çıkartılarak pişirilmeli
- Çiğ sebze ve meyveler iyi yıkanmalı, mümkünse kabukları soyulmalı
- Etler ve çiğ sebze, meyveler aynı anda hazırlanmamalı



