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EXPERIMENTAL CHLAMYDIOSIS IN WILD AND DOMESTIC LAGOMORPHS

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Abstract: Chlamydia psittaci (strain M56, the agent of epizootic chlamydiosis of muskrats and hares) was highly lethal for the snowshoe hare (*Lepus americanus*) following intravenous inoculation, whereas the agent was much less virulent for cottontail (*Sylvilagus floridanus*) and albino domestic rabbits (*Oryctolagus cuniculus*). Tissue titres of strain M56 were generally higher after 96 hr in the snowshoe hare than in the tissues of the other lagomorphs. Spleen, liver and bone marrow were apparently the chief sites of primary multiplication of strain M56 in the hare. Virulence appeared to be very host specific in that only strain M56 among the six chlamydiae tested was highly lethal for the snowshoe hare.

INTRODUCTION

A chlamydial agent, designated strain M56, was isolated during an epizootic in muskrats and snowshoe hares that occurred during 1959 to 1961 in Saskatchewan, Canada.[9] Strain M56 was identified as *Chlamydia psittaci*, and pathogenicity studies indicated high virulence, moderate toxicity, and broad host range.[11] The snowshoe hare, *Lepus americanus*, almost invariably succumbed to experimental infection whether inoculated by intravenous, subcutaneous, or oral routes.[5] Intravenous infection of hares with less than 10 mouse intracerebral LD_{50} was fatal. The inability of the snowshoe hare to cope with strain M56 infections suggested that the agent was poorly adapted to the host and may either represent a recent introduction into the hare population or a pathogen for which the hare has failed to develop an adaptive response because of some other interfering mechanism.

The present study was undertaken to determine the relative virulence of strain M56 for other lagomorphs and to determine if other chlamydial agents were as virulent for the snowshoe hare as strain M56.

MATERIALS AND METHODS

Chlamydiae

M56

After ten passages via yolk sac inoculation in 7-day-old embryonated hens' eggs, the infectivity titre reached $10^{7.6}$ $CELD_{50}$/ml and $10^{8.5}$ $ICLD_{50}$/ml in 3-week-old albino mice. Seed stock was prepared from the tenth passage as a 10% suspension of infected yolk sac. Tryptone broth containing 1 mg of streptomycin sulfate per ml was used as a diluent for infected yolk sac material.

Other chlamydiae

Stocks of other chlamydiae were prepared as 10% suspensions of infected yolk sac material using the tryptone broth-streptomycin sulfate diluent. Chlamydiae used were: 1) a turkey pneumonitis agent, designated JO (received from Dr. R. W. Moore, Texas A. and M. University, College Station, Texas);[1] 2) a parakeet pneumonitis agent, designated 6BC (received from Dr. J. E. Officer, Fort Detrick, Maryland);[6] 3) another turkey pneumonitis agent, designated T-31

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(characterized by Gale and Pomeroy);[2] 4) a mouse pneumonitis agent, designated Nigg (received from Dr. J. E. Officer, Fort Detrick, Maryland);[6] and 5) a trachoma agent, designated TW-3 (received from Dr. F. B. Gordon, National Naval Medical Center, Maryland).[3]

Experimental Animals

Snowshoe hares were trapped near Tomahawk, Wisconsin and cottontail rabbits (*Sylvilagus floridanus*) were trapped near Tomahawk or Madison, Wisconsin. To preclude mortality from extraneous causes, the hares and cottontails were adjusted to captivity by holding for at least two weeks prior to experimental infection. Albino domestic rabbits (*Oryctolagus cuniculus*) were obtained from W. Voss, Madison, Wisconsin. All animals exposed experimentally weighed between 1,800 g and 2,200 g, and were healthy and vigorous at the time of exposure.

Experimental Infections

Lagomorphs were inoculated via the lateral ear vein with a tryptone broth suspension of strain M56. After varying intervals, some animals were killed, and their tissues assayed for chlamydiae by intracerebral inoculation of 3-week-old mice (Tables 2 and 3).[5] The remaining infected animals were observed for one month, during which rectal temperature, body weight and survival were recorded.

In addition, an experiment was performed to determine the susceptibility of snowshoe hares to other chlamydiae (Table 4). Chlamydiae relatively avirulent for mice (TW-3 and Nigg) were used as well as highly virulent chlamydiae (6BC, T-31, and JO). After varying intervals, surviving hares were challenged with strain M56.

RESULTS AND DISCUSSION

All lagomorphs infected with strain M56 developed a febrile response and suffered weight losses of up to 200 g. The infection was not highly virulent for the cottontail and the domestic rabbit, but was fatal in all of the snowshe hares, 5 to 14 days post-infection (Table 1).

Strain M56 was present in most tissues of all infected lagomorphs, with higher titers detected in the reticuloendothelial and central nervous systems, skeletal and cardiac musculature, and kidneys of the snowshoe hares. Apparently the chief sites of primary multiplication of strain M56 in the snowshoe hares were spleen, bone marrow and liver.

Although the snowshoe hare was highly vulnerable to infections with strain M56, three virulent *C. psittaci* strains of avian origin (6BC, JO, and T-31) were capable of infecting the hare without producing

TABLE 1. Comparative mortality produced by experimental intravenous infections of lagomorphs with **C. psittaci,** strain M56.

Species	Infected [a]	Controls [c]
Snowshoe hare (*Lepus americanus*)	15/15[b]	0/4
Cottontail rabbit (*Sylvilagus floridans*)	1/10	0/3
Domestic rabbit (*Oryctolagus cuniculus*)	1/20	0/5

[a] Each animal was inoculated intravenously with 3.5 $\log_{10}$ weanling mouse $ICLD_{50}$ of strain M56.

[b] Numerator = total number of deaths; denominator = total number of exposed animals.

[c] Each animal was inoculated intravenously with 10% suspension of yolk sac in tryptone broth.

TABLE 2. Chylamydial titres in tissues of lagomorphs killed 96 hr after intravenous inoculation with **C. psittaci,** strain M56*.

Tissue	Snowshoe hare	Cottontail rabbit	Domestic rabbit
Blood at 40 hr	<1.0**	0	0
Blood at 96 hr	1.7	<1.0	<1.0
Spleen	5.7	4.3	2.7
Liver	5.0	4.3	3.5
Bone marrow	4.3	2.0	3.5
Popliteal lymph node	4.0	3.7	3.0
Cerebral cortex	3.5	<1.0	0
Base brain	3.0	0	1.3
Spinal cord	4.3	0	<1.0
Pituitary	4.0	1.7	1.0
Adrenal	4.3	3.7	1.5
Renal cortex	4.5	3.0	2.5
Lung	3.3	2.7	3.0
Heart muscle	3.3	<1.0	<1.0
Skeletal muscle	3.0	0	0

* Each animal received 3.5 $\log_{10}$ weanling mouse $ICLD_{50}$.

** Titres expressed as $\log_{10}$ weanling mouse $ICLD_{50}$ of chlamydiae per ml or gram.

TABLE 3. Chlamydial titres in tissues of snowshoe hares killed at various intervals after intravenous inoculation with **C. psittaci,** strain M56.

	Time of sacrifice (days post-inoculation)			
Tissues	2*	4*	8*	12*
Blood	0**	1.5	5.0	2.7
Spleen	4.7***	5.7	6.7	6.7
Liver	2.3	4.7	6.5	7.3
Bone marrow	3.7	4.3	6.3	6.3
Popliteal lymph node	<1.0	4.3	6.5	5.7
Cerebral cortex	0	3.0	5.7	6.0
Base brain	0	3.0	5.7	5.3
Spinal cord	<1.0	4.7	5.7	5.0
Pituitary	0	4.3	4.0	3.5
Adrenal	0	4.3	5.0	6.0
Gonad	0	<1.0	3.3	5.0
Kidney cortex	0	4.5	4.7	5.0
Lung	0	3.7	4.7	5.3
Heart	0	3.7	4.3	4.7
Skeletal muscle	0	3.3	4.7	4.5

* One adult hare inoculated intravenously with 2.0 $\log_{10}$ weanling mouse $ICLD_{50}$.

** 0 = no M56 could be demonstrated in undiluted blood or 1:10 suspension of tissue.

*** Titres expressed as $\log_{10}$ weanling mouse $ICLD_{50}$ of chlamydiae per ml or gram.

TABLE 4. Response of snowshoe hares to various strains of chlamydiae and subsequent challenge with strain M56.

Initial chlamydial infection					Subsequent challenge with strain M56 [d]		
Inoculum	Dose [a]	Fever [b]	Peak titres of agent in blood [c]	No. dead No. inoculated	Fever	Peak titres of agent in blood	No. dead / No. challenged
Yolk sac (controls)	0	—	0;0	0/2	+	3.6;3.6	2/2
TW-3 (trachoma)	2.3	—	0;0	0/2	+	4.0;5.1	2/2
Nigg (mouse pneumonitis)	2.5	—	0;0	0/2	+	2.1;3.4	2/2
6BC (parakeet pneumonitis)	4.6	+	0;0	0/2	+	2.1;3.2	0/2
JO (turkey pneumonitis)	6.5	+	<1;13	0/2	+	<1;<1	0/2
T-31 (turkey pneumonitis)	4.3	+	0	0/2	+	1.3	0/1

[a] Expressed as the $\log_{10}$ $CELD_{50}$ doses inoculated intravenously.

[b] + = elevation of rectal temperature by at least 1.5° C.

[c] Peak titre detected by daily bleedings for 10 days expressed as the $\log_{10}$ $CELD_{50}$ (for TW-3 and Nigg) or the $\log_{10}$ weanling mouse $ICLD_{50}$ for 6BC, JO, and T-31).

[d] Challenge dose = 3.5 $\log_{10}$ weanling mouse $ICLD_{50}$.

death (Table 4). Febrile responses occurred, but high titers of chlamydiae were not detected in the hares' blood. Infection with the three other avian *C. psittaci* strains induced immunity to the lethal effects of challenge by strain M56 whereas previous infection with *C. trachomatis*, strains TS-3 and Nigg, failed to induce any immunity to challenge. This reemphasizes the lack of cross-species immunity with *Chlamydia* although both chlamydial species share a group antigen.[12]

Speculations about the nature of the host-specific virulence of strain M56 in the snowshoe hare incorporate many factors.

Susceptibility of the snowshoe hare to environmental stressors was suggested by reports on "shock disease", a nontransmissible pathologic condition resulting in depletion of glycogen stores of the liver and terminal hypoglycemia which was associated with a decline in numbers of hares during the 1930's in Minnesota.[4]

Experimental infection of hares with strain M56 produced diffuse hepatic necrosis, altered liver function, depletion of liver glycogen, and terminal hypoglycemia.[5] In addition to the hepatopathy, strain M56 may produce a specific nutritional drain on the physiologic reserves of the hare by replicating to high titres in many tissues (Table 3), especially since chlamydiae are energy parasites, depending on their host for generating metabolic energy.[7] Overwhelming the host's defense mechanisms is an alternate explanation for the virulence of strain M56 for the hare. The importance of phagocytic and monocytic cells has been emphasized in resistance to chlamydial infections,[13] and the early marked concentration of strain M56 occurred in the reticuloendothelial system in the hare.

The extreme vulnerability of the hares to the agent of epizootic chlamydiosis represents an exceptional host-parasite relationship for the chlamydiae.[8] The relationship more characteristic of chlamydiae is one in which there are few overt signs and low mortality as in experimental infections of muskrats and mallard ducklings with strain M56.[10]

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