Introduction and history

How could you prove that a specific parasite causes a specific disease?

How could you determine what the vector for this disease is?

Why was a species of African trypanosome named after David Bruce?

What other parasites and diseases were named after specific scientists and why? Give at least three examples.

What did Ronald Ross observe in a mosquito and why did he conclude that this had something to do with malaria?

Compare Koch's postulates for proving that a specific parasite causes a disease and "Koch's" molecular postulates. Give an example for each.

"A parasite is an organism intimately associated with another organism who benefits at the expense of the other. "
Is this a completely or partially true definition?

What is a "fact" in science?
What are some examples of observations that are not "facts".

Associate the name with the discovery:
Carlos Chagas
David Bruce
Patrick Manson
William Trager
Ronald Ross
Donovan and Leishman
Leuwenhoeck
Discovered a new parasitic disease by first studying the insect vector.
Proved that the tsetse fly is the vector for African trypanosomiasis.
Attempted to create new theory that malaria was not due to "bad air" but to specific parasites in the blood.
Showed that blood-sucking insects are intermediaries in propagation of some parasitic diseases.
Cultured Plasmodium faliciparum axenically
Found trypanosomes in blood of cattle with Nagana.
Discovered the life cycle of Plasmodium parasites.
Observed amastigotes in spleen cells from a person dying of kala-azar.
First to see protozoa in microscope.
Development of apochromatic lens allowed what to be seen for first time?
Mitotic chromosomes

Which of Koch's postulates were utilized in the discovery of the cause of these diseases:

(1)African Sleeping Sickness

(2) Chagas Disease?

Chagas Disease

How can indirect immunofluoresce be used for either
(1) direct diagnosis of Chagas Disease
(2) indirect diagnosis of Chagas disease?
You are provided a specific antibody against T. cruzi trypomastigotes and epimastigotes and also you are provided live T. cruzi cells.

Associate a specific morphological type with a specific stage of the life cycle of T. cruzi. Draw a diagram of the T. cruzi cell of each type.
Trypomastigote
Amastigote
Epimastigote
Metacyclic trypomastigote

What are the requirements for a diagnostic procedure for a parasitic disease?

T. cruzi amastigotes reside inside a phagocytic valcuole.
True or False

What are the major differences between the life cycles of T. cruzi and L. major?

How can immunofluorescence be used for either
(1) direct diagnosis of Chagas Disease
(2) indirect diagnosis of Chagas disease?
You are provided a specific antibody against T. cruzi trypomastigotes and epimastigotes and also you are provided live T. cruzi cells.

Which if any of the diseases we have discussed (Chagas Disease, African Trypanosomiasis, Visceral Leishmaniasis, Dermal Leishmaniasis) could be considered "socio-economic" diseases?
Explain your answer.

Briefly contrast Chagas Disease and African Sleeping Sickness in terms of:
a. Life of the parasites in the vertebrate host.
T. cruzi
T. brucei
b. Preadaptations to the next stage of the life cycle.
T. brucei:
T. cruzi:
d. Acute versus late stage pathologies in vertebrate host (very brief descriptions).
T. cruzi:
T. brucei:
e. Effect of host immune response on parasites in vertebrate host.
T. cruzi:
T. brucei:

What has proved to be the best way to lower the incidence of Chagas Disease? Explain your answer.

a- Treating patients in the acute phase with a drug against the parasite

b- Flooding the region with sterile male bugs (what is required for this to work?).

c- Treating houses with insecticides that can kill kissing bugs.

d- Kill all animal hosts of the parasite.

African Trypanosomiasis

What experiments were performed or what were the observations that led to discovery of the insect vector for (1) nagana, (2) African Sleeping Sickness, and (3) South American trypanosomiasis? Who were the scientists involved in each study?

Poly A + RNA was isolated from the first wave of T. brucei long slender trypomastigotes from an infected mouse. The RNA was electrophoresed in an agarose gel and the gel was blotted to a nitrocellulose filter. The filter was probed with a labeled DNA probe to the 39 nt SL sequence. What type of pattern would you expect to see and why?

If you isolated total cell RNA from these cells and ran this in a gel which was blotted onto a filter, what would you expect to see if you probed the filter with a labeled DNA complementary to a portion of one specific VSG gene?

If you ran a pulse field gel of total DNA from these cells, blotted the gel and probed with the same DNA probe as in (2), what would you expect to see and why?

If you isolated DNA from the second wave of trypomastigotes in the mouse, what might the pulse field gel blot look like?

Describe the two types of human African Sleeping Sickness in terms of:
1. The parasites
2. The insect vectors
3. geographical location
4. course of the disease
5. diagnosis

You have isolated trypanosomes from a wild animal in East Africa and grown them as the procyclic forms in culture.
Without attempting to infect a volunteer, how would you determine if this strain is potentially infective to humans or not?

Why do some naturally occurring strains of *T. evansi* and *T. equiperdum* lack the kinetoplast DNA? Why does this not occur with *T. brucei*?

Compare and contrast South American trypanosomiasis and African trypanosomiasis in terms of:
1. Life of the parasite in the vertebrate host
2. Life in the insect vector
3. Preadaptations of the parasite to next life cycle stages in general.
4. Acute and late stage pathologies in the vertebrate host.
5. Effect of host immune response on the parasites in the vertebrate host

It has been stated that the tsetse fly is mainly responsible for the continued existence of wild animals in equatorial Africa.
True or false? Explain your answer

How was ApolL-1 shown to be the lytic factor in normal human serum that kills *T. brucei?*

Describe how ApoL1 was engineered to become a trypanosome-specific "drug". Explain the reason for each modification.

What was the evidence that (a) SL gene transcription uses pol II and(b) VSG transcription uses pol I.

What do you think could determine that only a single VSG expression site is expressed in a cell?

Does polycistronic transcription in T. brucei only involve pol II mediated transcription?

If you wanted to find a drug that specifically kills African trypanosomes and does not affect the host, what parasite pathways could you go after? List three such pathways and how you could block them.

How can human infective *T. brucei* survive in normal human blood?
Why do some naturally occurring strains of *T. evansi* and *T. equiperdum* lack the kinetoplast DNA? Why does this not occur with *T. brucei*?

Some strains of *T. equiperdum* and *T. evansi* have kDNA networks composed 10,000 minicircles of one sequence class and the specific sequence class varies from strain to strain. Explain how this is possible.

How do the diseases caused by *T. rhodesiense* and *T. gambiense* differ? Very brief answers.
***T. rhodesiense* -**
***T. gambiense* -**

How does ApoL1 kill *T. brucei* parasites in the human bloodstream?
Describe how ApoL1 was engineered to become a trypanosome-specific "drug". Explain the reason for each modification.

You have isolated trypanosomes from a wild animal in East Africa and grown them as the procyclic form in culture. How could you determine if the strain is potentially infective to humans?
How was phylogenetic profiling used to identify novel motility genes in *T. brucei?*

Leishmaniasis

Is the LPG surface coat of L. major involved in the parasite life cycle in the insect host?
Explain your answer.

What are metacyclic Leishmania promastigotes?

What is the receptor for attachment of the trypanosomes in the fly midgut and what is the biological role of this binding?

What are the subsequent physiological changes that allow the parasite to wind up in the proboscis of the sandfly?

Can you think of a method to isolate infective *L. major* metacyclic promastigotes from a culture?

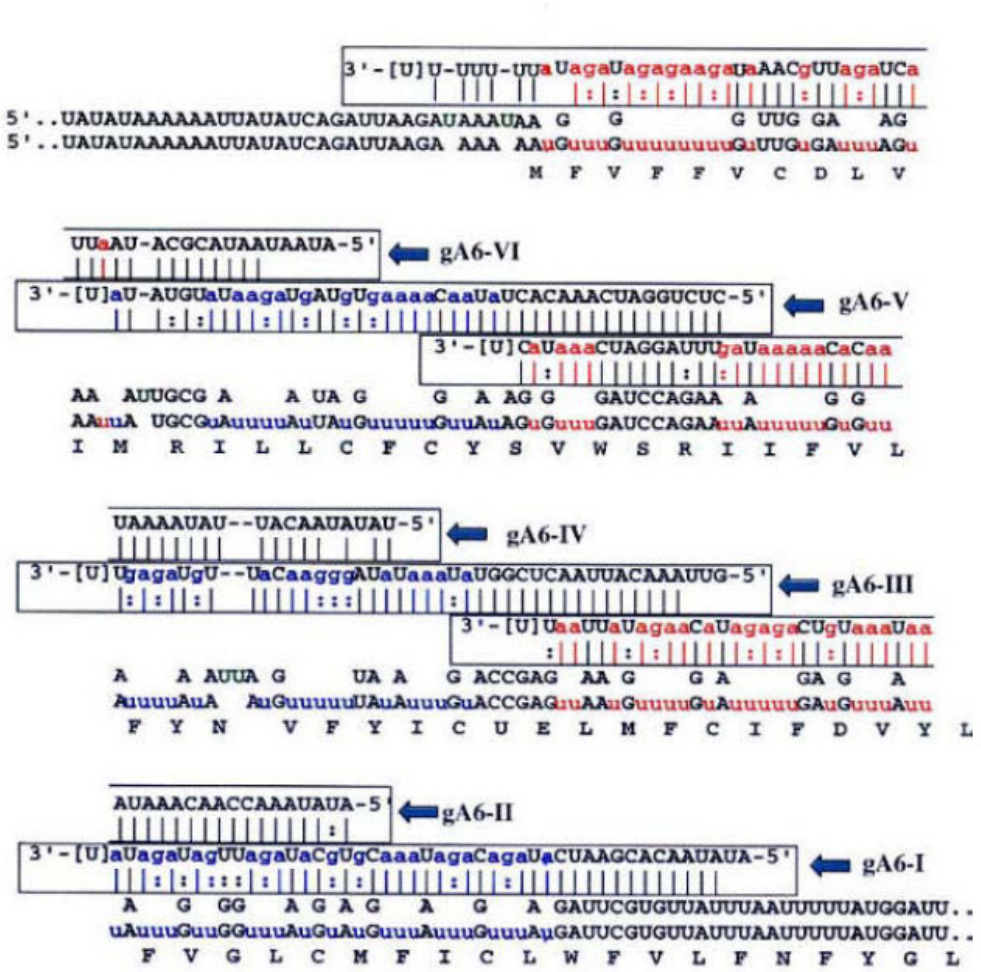
Why is it important to determine the specific species of *Leishmania* in the early stage of the infection even though the lesion will heal spontaneously?

Metacyclic promastigote *Leishmania* parasites regurgitated into the vertebrate host during the sandfly bloodmeal are taken up by (choose one):
a. macrophages
b. neutrophils
c. red blood cells
e. heart muscle cells
f. nerve cells

Associate the disease with specific parasite species or groups.
a. Mucocutaneous leishmaniasis
b. Old World Visceral leishmaniasis
c. Old World dermal leishmaniasis.
d. New World dermal leishmaniasis

RNA editing

G-U base pairs are shown with ":" and A-U and G-C pairs with "|".



You are given the following information about G-U, G-C and A-U base pairs.

G-C = -3 kCal/M
A-U = -2 kCal/M
G-U = -1 kCal/M

What do you think could possibly be a function of the G-U base pairs between the edited mRNA and the gRNA in RNA editing?

Describe the model for the change of pan-edited genes into partially edited genes in evolution.

Can there be more than one gRNA for the same block of editing? Why?

Describe the cut-splice model for RNA editing and indicate which enzymes have so far been identified.

Describe the evidence that the RET1 TUTase adds U's to the 3' end of the gRNAs and the RET2 TUTase adds Us to the editing sites.

What do you think was the initial event that created uridine insertion/deletion RNA editing in early cells?

How was the core editing complex isolated and the components identified? A labeled diagram is fine.

Kinetoplast DNA

Leishmania minicircles have a single gRNA gene whereas T. brucei minicircles have three gRNA genes. The computer simulations of minicircle sequence class plasticity were performed using the Leishmania minicircle model.
How would the simulations change if T. brucei minicircles were used.

Describe the Crithidia and the T. brucei models for kinetoplast minicircle DNA replication and segregation.

What could be the evolutionary reason to maintain this process of replication?

Genomics

What is a "DRG" (directional transcriptional unit)?

What is a "switch point"

Genes in subtelomeric regions of T. brucei?

What is "synteny"

How do you prevent large chromosomes from breaking when you run a pulsed- field gel?

What are possible reasons for the maintenance of synteny between the three parasites?

RNA interference and Micro RNAs

Compare and contrast siRNAs and microRNAs in terms of:
1. RNA processing involved in the formation of these RNAs.
2. Mechanism of action.

How was the "slicer" endonuclease activity identified?

Discuss the functional similarities between guide RNAs in trypanosomes, and snoRNAs and siRNAs in higher organisms.

General

If you wanted to find a drug that specifically kills African trypanosomes and does not affect the host, what parasite pathways could you go after? List three such pathways and how you could block them.

How could you prove that a specific parasite causes a specific disease?

How could you determine what the vector for this disease is?

The existence of a novel pathway in a parasite presents a target for selective intervention.
Explain and give an example.

Describe the life cycle of the parasite in the vertebrate host
1. ***T. brucei***
2. ***T. cruzi***
3. ***L. major***

Describe pre-adaptations of the parasites to the next life cycle stage.
***T. brucei*:**
T. cruzi

List five features of the biology of trypanosomes which were considered novel.