蚊が運ぶ病気と生態系の構造

Structure of Animal Communities and Transmission Dynamics of Mosquito Borne Diseases

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Hello, I am Yoshio Tsuda of the National Institute of Infectious Diseases.

As Prof. Murata mentioned during his introduction, I am first and foremost engaged in research on mosquitoes that transmit diseases to humans. These days however, just like Prof. Huffman who is working on simian malaria, and Prof. Murata who is working on avian malaria, I have also been working on animal malaria and particularly on the vectors that spread malaria.

I expect most of you have an image of mosquitoes as disagreeable insects that suck blood and spread diseases. But today, let's try to improve our understanding a little more about mosquitoes and the mechanisms by which they spread diseases, including the fact that not all species of mosquitoes actually spread diseases. And on that basis, let's explore how mosquitoes spread infectious diseases in animal communities including to humans. To begin with, I would like to talk more about what Prof. Murata mentioned in his introductory talk because, although mosquitoes do carry diseases, this need not be such an enormous problem. We should also be thinking about what the ecosystems that include mosquitoes can achieve.

In terms of content, I will be talking about the dilution effect that Prof. Murata introduced at the start.

Among the questions I am often asked about mosquitoes are "what good do mosquitoes do, and wouldn't it be better all round if they didn't exist?" Certainly, we tend to see only the bad side of mosquitoes. But let's take a closer look. This is a copy of a page from a textbook about mosquitoes, and it shows a mosquito larva, which is commonly known as a wriggler. This schematic diagram shows an image of a rice paddy. Perhaps most of you have not had this experience, but if you go to a rice paddy not long after the rice seedlings have been planted and look closely, you will see that there are a lot of wrigglers in the water. There are lots of rice paddies, particularly in Southeast and East Asia including Japan. And these places where wet-paddy rice cultivation is carried out are among the most important sources of wrigglers and mosquitoes.

At the same time, there are many different organisms also living in rice paddies. In particular, during the Japanese rainy season in June, when wrigglers are common, the fry of fish such as killifish, crucian carp and loach are emerging from their eggs. These young fish rely on wrigglers as a major part of their food intake. Water fleas also feed on the newly hatched mosquito larvae and other insects also use them as a food source. The number of wrigglers that hatch each year is enormous, and they can be considered an important food resource for many other creatures.

While we are focused on the image of the adult mosquitoes that come around to suck our blood, these creatures are in fact eaten by a host of natural predators such as birds, bats, frogs, spiders and dragonflies that prey on insects. So they are an extremely important food source for natural predators. Additionally, the wrigglers feed on the straw from the previous year's rice crop found in the waters of rice paddies. In so doing they help break this material down.

Given this other role as scavengers they are a vital component of the ecosystem and their absence would cause problems.

Of course, mosquitoes do not only live in rice paddies and, worldwide, there are about 3,000 known species. In Japan alone, about 112 species exist in the wild. These various species of mosquito have different breeding habits and their wrigglers hatch in different places. I'd like to talk about that next.

Mosquitoes live in a wide range of habitats, but for today's talk I will show you some data from the rice paddies of Izumo in Shimane Prefecture. I'm sure everybody here today has heard of Izumo, which is around here on the map. Here is Lake Shinji, and here is the rice paddy zone where the data was gathered.

The reason why this particular area was chosen for a survey is that it is famous for the enormous number of migratory waterfowl that visit, as you can see from this photograph taken in October of the survey year. These birds overwinter in Izumo but there are other species of birds that are summer visitors. Because this small area attracts so many migratory birds, if individual birds carrying infectious diseases arrive, it is easy for the infections to spread. For instance, a mosquito biting an infected bird and sucking its blood also takes in disease-causing agents, or pathogens, present in the bird's blood. Later, this same mosquito may go on to bite another bird or even a human being, thereby infecting them with the pathogen too. My main work involves investigating these kinds of infection routes and the amount of risk they entail. I survey mosquitoes in various places where migratory birds congregate, and Izumo is one of these places.

This figure is small so it may be difficult to make out. It is a relief map, and just here, where you can see a grid pattern, is the rice paddy zone. And naturally, because rice paddies need irrigation, there is a river. Here you can see the characteristics of Izumo. This is the hilly area behind the rice paddies, and you may be able to see some small ponds dotted about, which are marked in blue. These are irrigation ponds. Even today some of them are still in use but in the old days water from all of these reservoirs was used to provide irrigation water for rice growing. These days a different water supply system is used so most of the irrigation ponds are no longer used. For the most part nobody takes care of the unused ponds so they have become places where the wrigglers, the mosquito larvae, hatch and grow. This is a photo showing one of these ponds. The water here is an ideal breeding ground for wrigglers and so I select places like this for catching mosquitoes.

The surveys are carried out in this kind of hilly area and also along riverbeds in locations where there are a few trees growing up from the riverbed.

Within the rice paddy areas, capturing mosquitoes inside the paddies themselves is difficult. So, because in most rice-growing communities there is a Shinto shrine surrounded by thick growing vegetation, I try to capture mosquitoes there using traps.

Here in Izumo, there is another interesting survey spot known as the "Sagi Yama" which translates as "heron mountain" in English. In summer, it attracts a great many herons, but various other kinds of birds also congregate there and build their nests, so there are a lot of nests concentrated in the same location. Such places are commonly called "sagi yama" in Japan, so when I was informed that there was a heron mountain in Izumo, I decided to collect mosquitoes there. Today, looking hard at my own slides, I've noticed that although the subject of today's talk is "mosquitoes", there is not a single mosquito image among them. Although I said I was going to talk about mosquitoes this talk is actually less concerned with the mosquitoes themselves.

This is the mosquito species Culex tritaeniorhynchus which is a vector in transmitting Japanese encephalitis. Japanese encephalitis has been the biggest single infectious disease problem in Japan. These days there are almost no patients with the disease, but there is still a problem in that a number of people do become sick every year who appear to be infected with it on first glance. These mosquitoes were captured in the vicinity of a pond. You can see that there are mosquitoes of different colors, which means that they belong to different species. So from this you can see that there are various species present. About one quarter of the individuals collected were of C. tritaeniorhynchus. At the shrine however, the mosquitoes collected were of only three colors so the total number of species was very small. Again, C. tritaeniorhynchus accounted for about one quarter of the individuals. In riverbed areas we come across individuals of a few other species, but in these places too C. tritaeniorhynchus is extremely common. This also shows that we capture different kinds of mosquitoes in different places.

Perhaps, what we can catch depends on what kinds of mosquitoes live in what kinds of places. But, more than that, because movement and behavior varies between different species of mosquito, each species tends to live in the places it finds convenient and easy to live.

To find out what a heron mountain is like, let us take a look at the data obtained from the heron mountain in Izumo. This particular heron mountain is not such a big one. When I talked to some locals at the start of the survey they told me that it was certainly big in the old days. When I went to the site, the first thing I noticed was a strong smell. There were lots of bird droppings lying around and the smell was overpowering. It was also very noisy. These birds tend to gather in places where it is difficult for people to walk. While the number of birds at this site was not particularly large it was a proper heron mountain in which night herons, as well as little herons and cattle egrets, had all built their nests en masse. Beneath this heron mountain, among the vegetation and the whitish bird droppings, if you dip into the soil where you can see these little channels, you can capture the mosquitoes that suck the blood of various animals.

Here is a different heron mountain that I visited in Tokushima Prefecture during the following year. This is a huge heron mountain that is literally covered in nests. The birds make their nest at heights from between two meters to five or six meters. This nest is positioned approximately two meters above the ground, which is why I was able to take a photograph of it. Here and there in the heron mountain there are fallen trees, and lots of mosquitoes rest on the tree branches.

This species of mosquito was captured from a heron mountain but I am not going to talk about this particular species today. In total I have captured about 16 species of mosquito. Now I would like you to take a look at this red area. This is a mosquito that has sucked blood. It has a partially full belly but, as I'm sure you know, mosquitoes can keep sucking blood until their belly becomes really huge. That one there is a full-fed mosquito. Those with only partially full bellies have only sucked a little blood. Perhaps the host bird moved and caused them to stop while they were feeding. This one has a belly half full with blood while the other half has already been digested. When the blood has been half digested, the mosquito produces eggs while continuing to digest the remaining blood. In this case, the eggs are filling up about half of the belly space. The condition of this next one is that all the blood has been digested and the belly is bulging with eggs. This one has a flat belly because it has not yet sucked any blood at all. We catch different mosquitoes exhibiting a variety of physiological conditions.

My main subject today is the analysis of the blood that these mosquitoes hold. After bringing back the mosquitoes, I ask someone to assist me with the things I can't do myself such as removing the animal blood from the mosquitoes and extracting DNA from the blood. By obtaining the DNA base sequence from the blood and checking this against similar base sequences, we are able to identify the species of animal from which an individual mosquito has sucked blood.

Here, from the data obtained in Izumo you can see a lineup of the animal species we found to have been the sources of the blood taken from the mosquitoes analyzed. Here are the species of mosquitoes. Here, surrounded by a thin green line are the birds which include great egrets, cattle egrets and intermediate egrets. These mosquitoes were taken from a heron mountain so there were a lot of heron and egret samples but we also found some sparrow blood. Here we have some mammal blood samples including blood from raccoon dogs and Japanese sika deer, which doubtless live in the area, as well as from mice, etc. Then there were frogs, such as tree frogs. And here at the bottom we have grass lizards.

As you can see, mosquitoes suck the blood of many kinds of animals ranging from birds to reptiles. Here, I have also split the mosquitoes into several groups. The red group is the group that feeds on frogs. This kind of mosquito is the most familiar one to most of us here today. It's the kind that buzzes in your ear during the night and wakes you up with an itchy mosquito bite. The mosquitoes that have this red part here mostly feed on birds. This group has a strong preference for bird blood. The next group is known as the striped mosquitoes. In Japan, when we go out in the summertime and sit on a shady bench in the park, we are likely to be bitten by a mosquito in no time. The mosquitoes lying in wait for us are the striped mosquitoes. As a group, they limit their blood-sucking activities to mammals in almost all cases. This next one is of the species Uranotaenia novobscura, known in Japan as "chibi ka" meaning "small mosquito". And indeed they are about twice as small as most other mosquitoes. They are particularly fond of feeding on frogs. The reason I was able to capture quite a lot of this particular mosquito species at this heron mountain was, as you might expect, because they came there to feed on the frogs. This next type is vary rare, but there are also mosquitoes that suck the blood of snakes, lizards, etc.

Many mosquitoes of the genus Culex, known as house mosquitoes, are extremely fond of birds and also feed partially on humans and other mammals. Moreover, there are species that feed almost exclusively on mammals, and, similar to Uranotaenia novobscura, there are species that like feeding on frogs, although they also drink the blood of mammals occasionally. And then, there are more adventurous eaters that like to feast on the blood of reptiles and amphibians. So all in all, there are different mosquitoes that follow a wide range of blood sucking patterns depending on the species or group to which they belong.

Despite all this variety, basically all the different mosquito species have a lot in common. First of all, if we consider how their so-called blood sucking patterns are decided we find that these patterns are determined by genetic factors. It turns out that house mosquitoes have the strongest disposition to suck the blood of birds while striped mosquitoes have a strong disposition to suck the blood of mammals. In this sense, their behavior is genetically determined.

However, that isn't the end of the story. For example, on a heron mountain, where there are lots of herons and egrets, it is clear that the mosquitoes will tend to suck the blood of these birds. Where there are herons, the mosquitoes nearby will try to drink their blood and where there are no herons, the local mosquitoes will try to drink the blood of other animals. If there are no birds, they will feed on mammals such as raccoon dogs or on other animals, depending on which species are present and in what ratio to each other. Also, some mosquito species prefer to feed at night and others prefer to feed during the daytime. Generally, in my understanding, the overall balance of biological, behavioral and genetic factors determines the blood-sucking pattern. In particular, whether or not it is easy to suck the blood of a particular kind of host is determined by ecological factors. For instance, it is easier to suck the blood of animals that do not move around at night.

Looked at in this way, the heron mountain results I outlined earlier can be much better understood. This slide is very difficult to understand, but let's take a quick look at it. Up to now I've been talking about mosquitoes, but now I'd like to talk a little about the diseases that are transmitted by mosquitoes. And then, to finish up, I will talk about how mosquitoes spread these diseases among groups of animals.

Mosquitoes act as vectors in transmitting a variety of pathogens. Among the diseases spread by mosquitoes that infect humans are malaria, dengue fever and Japanese encephalitis. As for animal diseases, mosquitoes carry avian malaria, simian malaria, canine filariasis, etc. The mosquitoes' capacity to spread infectious diseases is determined by a number of properties. The first of these is their affinity with the pathogen in question, although not all species of mosquitoes can transmit disease. For instance, Culex tritaeniorhynchus is the most proficient mosquito at transmitting Japanese encephalitis. This is because the Japanese encephalitis virus does not propagate in the bodies of other mosquito species. So the capacity to spread infection is partially dependant on the compatibility between the host mosquito and the pathogen it carries.

And then, in the first place, there is the question of what kinds of animals a given species of mosquito prefers to feed on. Another important property is the lifespan of individuals of a given species. And lastly, there is the question of how many intervals of the species are produced. Ultimately, the capacity of a group of mosquitoes to spread disease depends on the cumulative effect all of these properties.

Today, I am particularly interested in two things, namely, 'affinity for pathogens' and 'blood-sucking preference'. These two things determine the combination of vectors - the mosquitoes that carry infections - and the animals that become the hosts for the particular pathogens carried. So this is the most interesting part for me.

This slide may also be a little difficult to understand. The uppermost part of the slide shows a mosquito's body cut in half longitudinally. Here is the head, this is the thorax, or chest, and here is the abdomen. The wings would be about here and the legs would be here, but these have been removed.

First, if we talk about malaria, for instance, whether it is simian malaria or human malaria or whatever, when a mosquito ingests the blood of an animal infected with malaria, the blood goes into a part of their abdomen known as the midgut. After that, the malaria pathogens in the blood form lump-like swellings called oocysts on the outside of the midgut. The pathogens develop into a form known as sporozolites inside the oocysts until they eventually burst out in the form of worm-like vermicules. In this form, the sporozolites enter the mosquitoes' salivary glands. Then, when an infected mosquito sucks the blood of another animal, the sporozolites are transferred inside the animal along with the saliva. This is how malaria is spread.

As is written here, when a mosquito sucks the blood of an infected animal, it takes about 12 days for the sporozolites to enter the salivary glands. So until this amount of time has passed, the mosquito cannot propagate malaria. In this respect, the lifespan of the mosquito is very important.

Next, I would like to talk about how mosquitoes spread malaria among groups of animals, and my talk will cover basically the same subject as the dilution effect that Prof. Murata explained at the beginning.

For infectious diseases, groups of animals usually include those who become hosts for the pathogens and those that do not become hosts. Or in other words those that do not become infected with the disease in question. Mosquitoes pick up pathogens from infected host animals and transmit them to other animals when they bite with the result that the disease is spread or "amplified". This is known as the 'amplifier concept'. In short, a relationship exists between the mosquitoes and their hosts moderated by the activity of blood sucking.

As for animals that do not become hosts, for instance, if a mosquito carrying avian malaria bites an animal that cannot be infected with this pathogen, the disease will not be spread and so this will not aid the pathogen' s propagation. The more often this happens, the more the transmission of the disease will be diluted.

Ultimately, this balance determines whether or not an epidemic will occur. If the mosquitoes come into contact with large numbers of animals that can serve as hosts, the amplification effect causes an epidemic to spread. On the other hand, if the dilution effect is larger, then the scale of epidemic will be limited. Unlike ticks, mosquitoes are good at choosing their hosts, and so the mosquito blood-sucking pattern is very important for determining the balance of whether or not an epidemic will continue to spread.

Now, I would like to share with you some data taken from an article on simian malaria that was written over 50 years ago. As Prof. Huffman explained earlier, simian malaria has recently become a major problem in South East Asia, and half a century ago similar research was carried out on this disease. Here in a forest, on the branches of larger trees, platforms were set up in various locations with cages on them with monkeys placed inside. The mosquitoes that came to suck their blood were then captured. This research was conducted in 2007, but exactly the same kind of research was carried out 50 years ago. In some cases, humans sat in the cages to attract mosquitoes.

I really like this data. These mosquito species were captured in a coastal mangrove forest. Using the same method the researchers captured mosquitoes from the lowland swamp behind the mangrove forest, and from the hill country behind the swamp. Naturally, the mosquito species they found in these differing places were different. The problem was that there were spherical oocysts on the outside of the mosquito midgut and there were sporozolites in their salivary glands. This was what the data indicated.

We know that this part marked in red refers to simian malaria parasites. As the data shows, it means that the species of mosquitoes that were captured coming to bite the monkeys were carrying simian malaria. As I explained earlier, there is a connection between this and the “amplification” of malaria. What the green part shows is that, as the researchers worked hard to establish, these chevrotains, or mouse deer, become infected with this type of malaria. I don’t really understand this blue part very well. But at the time, and even now, we are unable to classify all the things that emerge from the bodies of mosquitoes merely by their shape. Even so, from the shape of this one, I can tell that it is certainly not simian malaria. And looking at this one, I would guess it to be avian malaria. This means that, although these mosquitoes came to feed on monkeys, the malaria parasites they carried were totally unrelated to monkeys. So this contact between mosquitoes and monkeys was an example of a full dilution effect.

With regard to simian malaria, we know the following things. We conduct research into avian malaria, and when avian malaria comes up as a subject of conversation, we can’t avoid the feeling that this disease is happening in places where people have totally different lifestyles to Japan, such as South East Asia and Africa. But even so, when I was actually researching avian malaria, I found very similar stories. I would like to talk a little about this to close my talk.

This is a park in Tokyo where I conduct surveys. It is a rather large park. There are lots of trees growing, and I am always capturing mosquitoes in this area. In places where this sort of vegetation grows I can capture mosquitoes by sweeping a net about. The place may be in urban Tokyo but I am still able to find 11 species of mosquito here. This is a red house mosquito (Culex pipiens pallens) and this part surrounded in red is the blood sucking part. My analysis was centered on individual red house mosquitoes that had sucked blood.

My analysis found that between 3% and 5% of the mosquitoes that had not sucked blood were carriers of avian malaria. In the case of mosquitoes that had sucked blood, an analysis of the abdomen revealed that 18.6% were carrying avian malaria, while about 3% seemed to be carrying it in their chest or salivary glands. So out of every 100 of these mosquitoes, we can be sure that several are carriers of avian malaria.

This is a survey of the animals on which the mosquitoes I captured had been feeding. Those surrounded by the dotted lines are all birds. There was only one human blood sample, meaning that out of the 220 mosquitoes surveyed, only one had bitten a human. The above three species, namely jungle crow, sparrow, and great tit, accounted for 83.4% of the samples obtained. This part in red shows the numbers of avian malaria samples obtained. The ratios were 29%, 21% and 40%, respectively. So in all probability, these three species of birds can be considered as host species for avian malaria in Tokyo. I think that under this situation, the fact that so many mosquitoes feed on these host birds creates a great many contact points for amplifying the spread of avian malaria and gives little opportunity for dilution effects to operate.

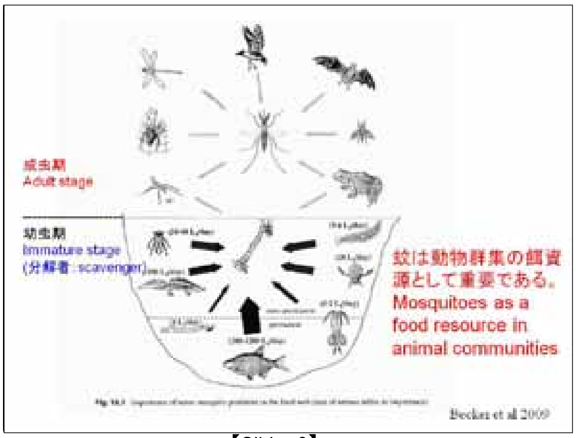
Actually, the species of mosquitoes that feed on birds change according to the season. For instance, the numbers of mosquitoes that feed on crows are low in May but increase gradually through June, July and August. And in line with this increase in numbers, the ratio of mosquitoes carrying avian malaria parasites also changes.

This chart shows data on various species of birds. We looked at three species - crows, sparrows and great tits. The vertical axis shows the risk of avian malaria infection for each species. For the sparrows, marked with a white circle, the risk of becoming infected with avian malaria from a mosquito bite is high at the start but becomes lower in the summertime. The great tits tend to be bitten little by little throughout the period but there is no particular time when lots of great tits are being bitten. So the risk of this species being infected with avian malaria, while present, is very low. In the case of the crows, which have the biggest problem, as the summer progresses through June, July and August, the mosquitoes come to feed almost exclusively on crows. Therefore the risk of these birds contracting avian malaria is very high. In the course of our research, we found out that another such high risk period occurs around October.

Put more simply, when we examined the red house mosquitoes captured at the park, we found evidence of blood from avian malaria host bird species such as crows, sparrows and great tits, and also of non-host bird species. In this case, a single mediator species and several host species are involved. When mosquitoes feed more on non-host species, we can expect the dilution effect to be favored, but when they feed more on host species we can expect to see the amplification effect happening. In this way, the overall propagation ratio of pathogens is influenced by how many host and non-host animals are available to the mosquitoes.

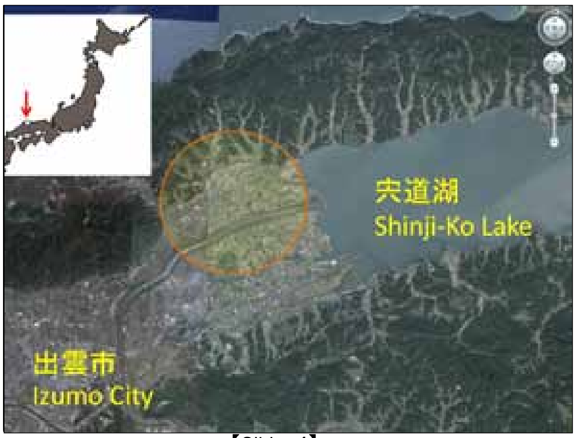
Moreover, in the survey area there were other animals besides birds and other mosquitoes than the species surveyed. We had to limit the species of mosquitoes in the survey, and so we chose those species that fed most often on birds. These included the red house mosquito that I have been discussing. On this occasion we also surveyed a member of the striped mosquito family, but we found that these mostly sucked the blood of mammals. Although on this occasion we targeted avian malaria, if we understand the conditions under which avian malaria is sustained in more detail, we will have a better idea about how this disease, which is transmitted to wild animals by mosquitoes, is able to exist.

【Slide 1】



【Slide 2】

【Slide 3】



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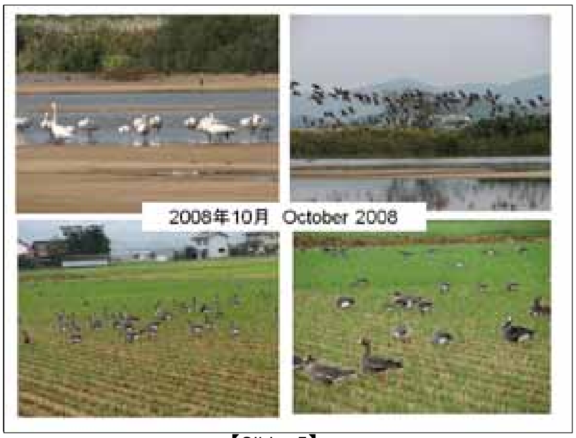
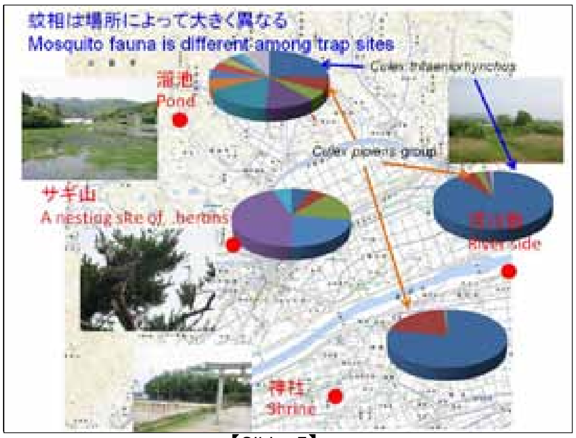


【Slide 5】

【Slide 6】



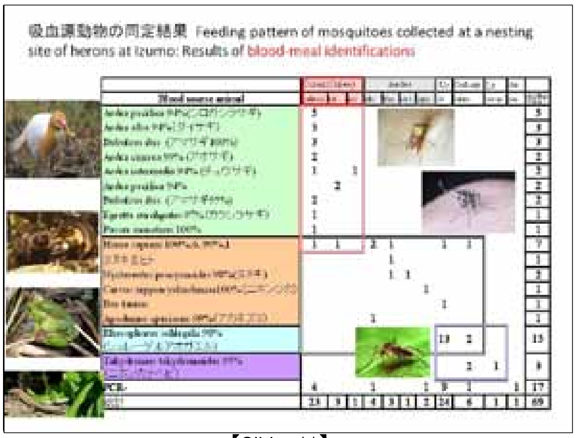
【Slide 7】

【Slide 8】

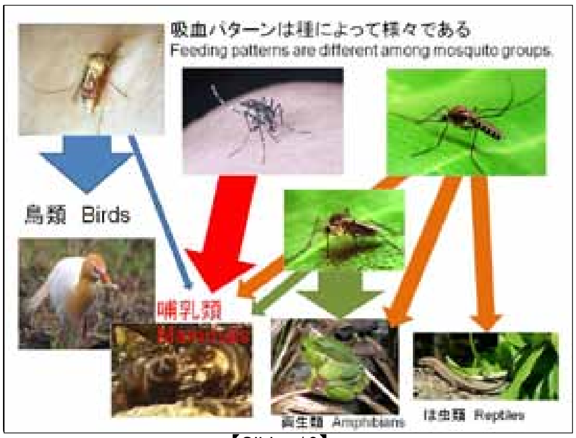
【Slide 9】

Species	full	partial	half gravid	Gravid	unfed	♀TTL	♂TTL	Total
1 Ae. albopictus		4			40	44	15	59
2 Oc. japonicus			2		44	46	10	56
3 Ae. flavopictus	1	2			15	18	22	40
4 Cx. pip. gr	8	1	11		6	26	2	28
5 Cx. [illegible]	1		2	1	11	15	9	24
6 Ur. novobscura			7	5	3	15	8	23
7 Ae. vexans	1			3	10	14		14
8 Tr. bambusa					4	4	8	12
9 Ar. subalbatus					4	4	5	9
10 Cx. bitaeniorhynchus		2	1		2	5	1	6
11 An. sinensis			1		1	2	3	5
12 Cx. (Culiciomyia) sp.					3	3	1	4
13 Cx. orientalis	1					1	1	2
14 Cx. rubithoracis					2	2		2
15 Cx. hayashii							1	1
16 Cx. tritaeniorhynchus					1	1		1
Total	12	9	24	9	146	200	86	286

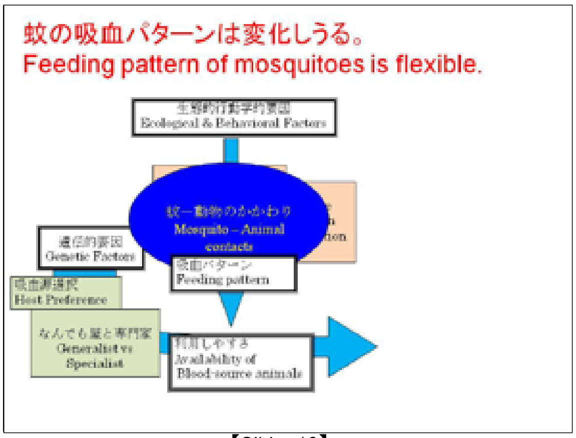
【Slide 10】



【Slide 11】



【Slide 12】



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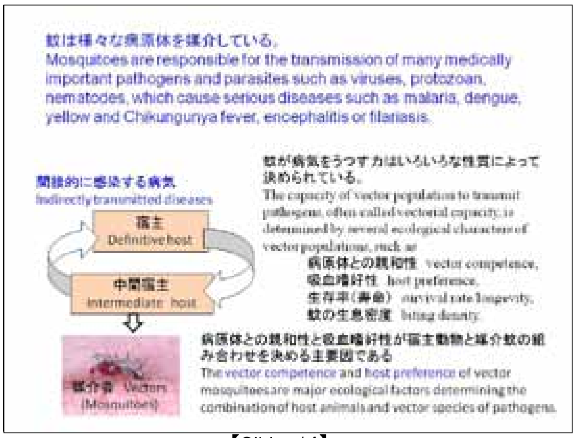

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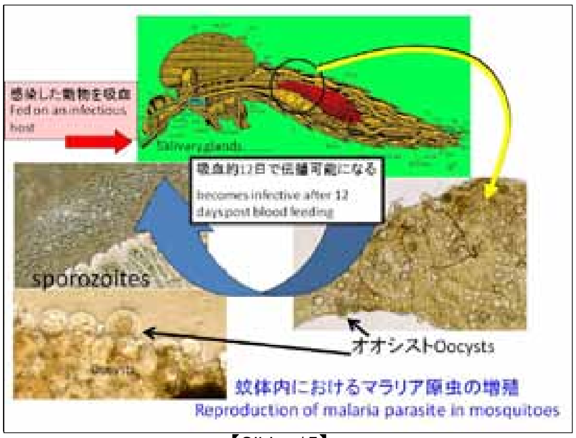

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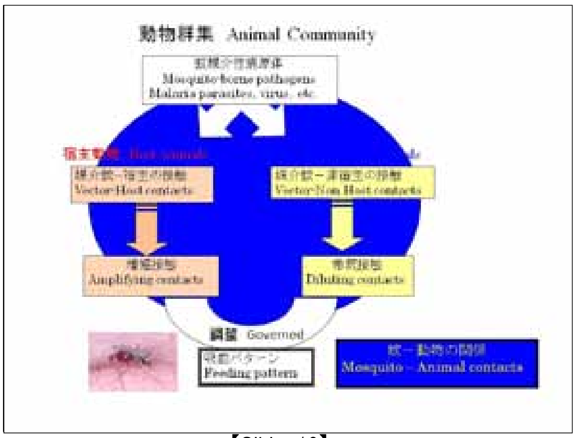


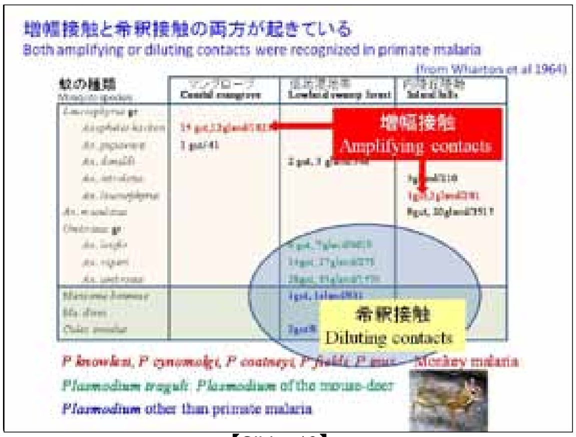
【Slide 16】

【Slide 17】

【Slide 21】

【Slide 18】

【Slide 22】



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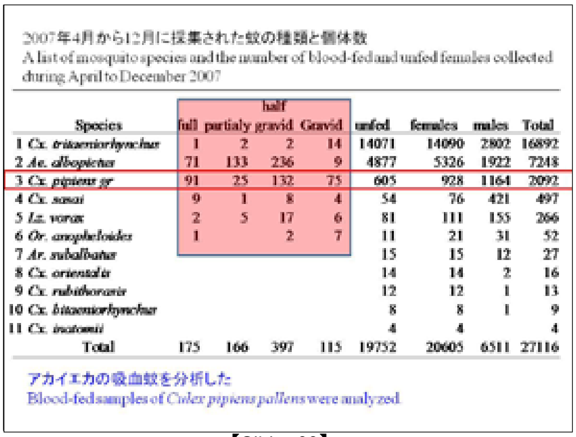
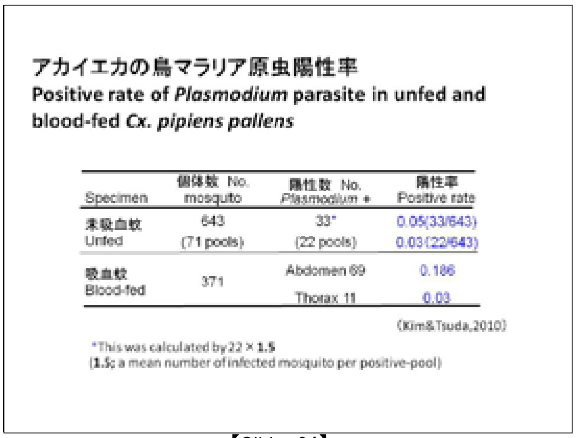


Species	full	partialy	half gravid	Gravid	unfed	females	males	Total
1 *Cx. tritaeniorhynchus*	1	2	2	14	14071	14090	2802	16892
2 *Ae. albopictus*	71	133	236	9	4877	5326	1922	7248
3 *Cx. pipiens gr*	91	25	132	75	605	928	1164	2092
4 *Cx. sasai*	9	1	8	4	54	76	421	497
5 *Lt. vorax*	2	5	17	6	81	111	155	266
6 *Or. anopheloides*	1		2	7	11	21	31	52
7 *Ar. subalbatus*					15	15	12	27
8 *Cx. orientalis*					14	14	2	16
9 *Cx. rubithoracis*					12	12	1	13
10 *Cx. bitaeniorhynchus*					8	8	1	9
11 *Cx. inatomii*					4	4		4
Total	175	166	397	115	19752	20605	6511	27116

【Slide 23】

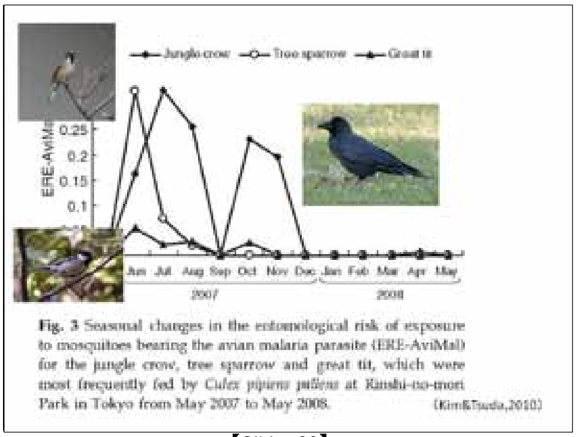
【Slide 20】



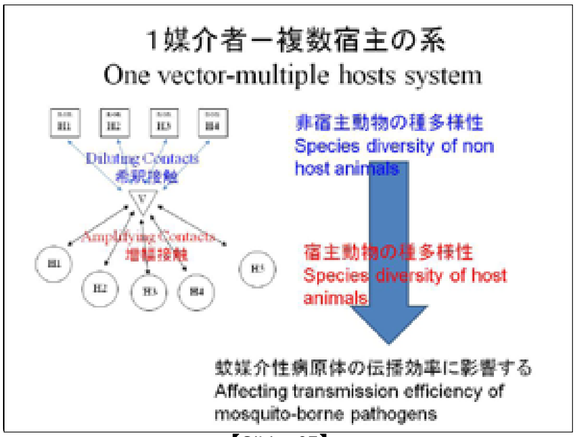
Specimen	個体数 No. mosquito	陽性数 No. *Plasmodium* *	陽性率 Positive rate
未吸血蚊 Unfed	643 (71 pools)	33* (22 pools)	0.05(33/643) 0.03(22/643)
吸血蚊 Blood-fed	371	Abdomen 69	0.186
		Thorax 11	0.03

【Slide 24】

【Slide 25】



【Slide 26】



【Slide 27】

【Slide 28】