Applied Environmental Microbiology

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Lecture - 29

Virus II

Dear students, in the second lecture about viruses; especially when is a protest to environment and public health, we will be talking about a first viral evaluation and how virus played role in evolution of DNA based life and then will be talking mostly about pathogenic viruses because that is very important for public health and here.

I would what like to mention to you that then we will be talking about wastewater treatment and then will be talking about water treatment I briefly go over water born viruses and I will go for bacterial fage. So, we cover the environmental health later, but for today our focus would be mostly on public health and little bit as I mentioned in the previous lecture we will also be talking about viroids in briefly about prions.

So, let us get started one of the most promising evolutionary theories that has been proposed recently and then good evidence has been gathered in its favour is that life started with RNA basis. So, earlier we did not have this additional step of DNA that required to be transcribe into RNA, but there was just RNA present and then proteins will translated from RNA and life continued, right.

So, all the protoviruses are believed to be RNA based viruses even the initial cells bacterial cells or archaea cells they are assumed or believe to be RNA based life forms now dear students if you have attended the previous lectures really well you must know that RNA is not a very stable genetic element it had it is quite short lived. In fact, some RNA messenger RNAs have half life of few minutes and some of them up to few hours, but DNA can last lived long.

So, we note here that our initial life of RNA base. So, they did not have to spend lot of energy in the transcription process and could may proteins directly; however, it is now proposed that the; it was the RNA based viruses that eventually developed DNA specific enzymes that allow them to become DNA virus. So, initially they were its hypothesize

with some evidence that initially were RNA virus and then this DNA base enzymes allow them become DNA viruses, but with the uracil. So, they still have held onto the uracil that is typical of r n, but that was still DNA based on virus and then they got rid of uracil and had time in which is more stable.

So, than they became DNA based viruses with a timing unit know when these DNA base virus they infected other cells. So, remember they can under go to a process they undergo lytic pathway where they replicate inside the cell they make proteins and more genetic material and then they break of the lies the cell membrane and they are free not to go and infect other host cells in the lysogenic pathway they becoming interior are part of the cellular chromosome and they replicate usually and they just remain silent until they move back to the lytic pathway.

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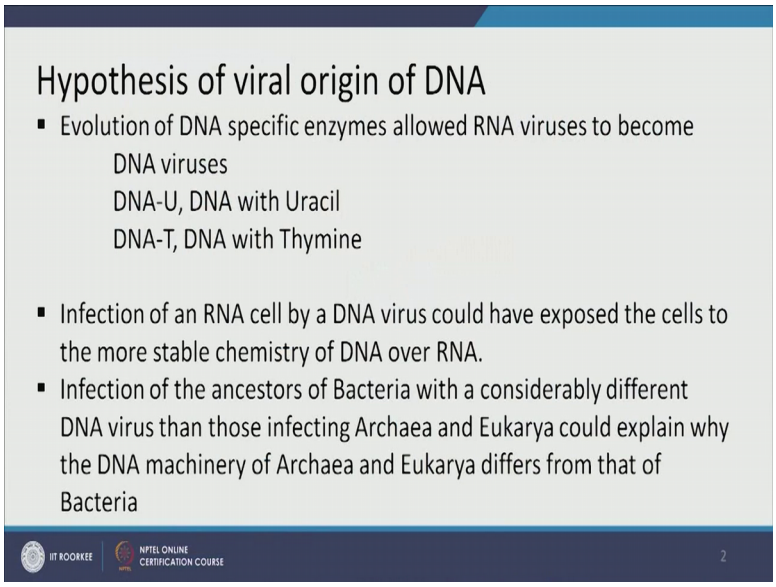

But, so, it is believed that when these DNA viruses they infected the cells and they underwent the lysogenic pathway not the lytic pathway the cells discovered that some part of the genetic material was very stable. So, the RNA base genetic material would degrade very fast, but then this viral genetic material the DNA based viral genetic material would last longer and thus infection of an RNA cell by DNA virus cloud have expose to the cells to the most stable chemistry of DNA over RNA.

Now, when the ancestors of bacteria or the proto bacteria they are they were probably infected by very different kind of viral DNA than the ones at ancestors of archaea and

eukarya were infected and that is why we see that the bacterial mechanism and metabolism at a times very different than archaea and eukarya. So, archaea and eukarya very close to each other in some several functions. So, maybe it is just that the prototype of bacteria was infected by different DNA based viruses and that is the cellular function machinery somewhat different already.

So, now let us now that I have cleared this about the latest theory and evolution of virus let us move on to tumour virus we briefly talked about them in the previous class, but let us say could look at tumour viruses.

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So, what I am going to show you is actually a virus called polyoma viruses and this induces tumour in human beings.

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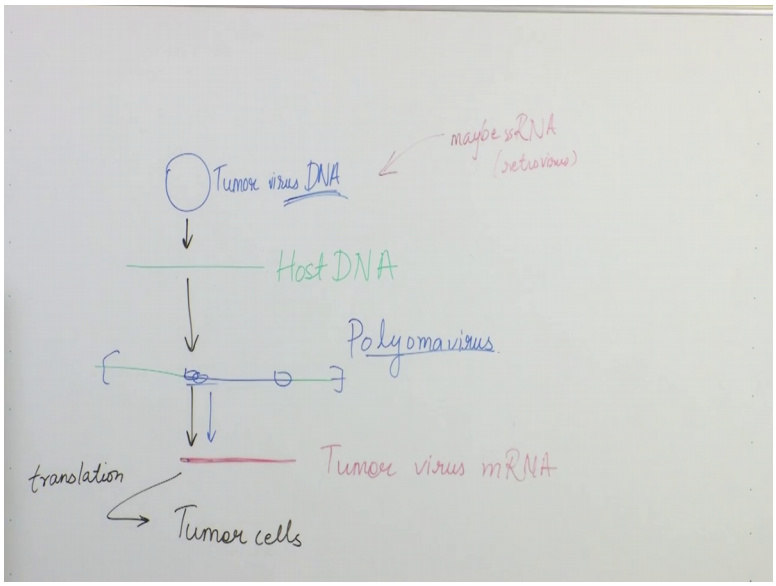

So, these is polyoma virues, and let us look at how polyoma viruses enters the cell and causes us a machinery to convert into tumour cells.

So, you have this polyoma virus which is a circular DNA based virus. So, this is tumourd virus which is DNA based and most it is injected into the cell what it does is it undergoes lysogenic pathway not a lytic pathway. So, basically if the green is the host cell it will integrate itself into the host d n a. So, now, that is integrated into host DNA whenever this portion of the DNA of the host cell would be transcribed it will make m RNA for the entire portion. So, because DNA this will have its n start code n and stop code on and rebuson binding and all those thing all those things it will make an m RNA which will be a tumour virus m RNA. So, what was the DNA a potential for making certain proteins or potential for causing certain activity in the cell now becomes a message m m RNA.

Now, this m RNA if the cell is healthy and working fine and does not identify this to be a for in genetic material then it will translate it into it will translate it into proteins that will convert a healthy cell into tumour cell. So, basically healthy animal cell can we converted into tumour cell by a lysogenic pathway by tumour viruses such as poli oma virus now there are certain viruses like in the previous lecturer I talked about it 2 virus are using the RNA viruses.

So, does not have to go undergo it is in have to be tumour virus based anyway it can be single stranded RNA virus to him it might also causes tumour I talked briefly about

retrovirus you know coming to retrovirus replication. So, remember retroviruses other viruses that that have single stranded RNA in them in the previous lecture I talked about how retroviruses survive in the cell and how they are replicate themselves; so, let us going to little bit more detail about retrovirus replication using reverse transcriptase.

So, basically in the virion RNA there are at the end is there direct repeats.

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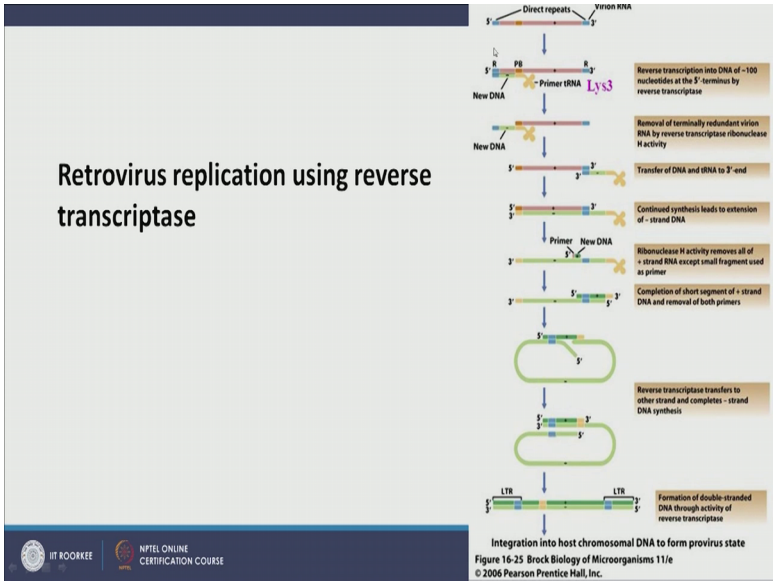

And then reverse transcription happens and we start from the five prime end and there is this primer t RNA here. So, the terminally redundant virion RNA is removed by the reverse transcriptase ribonuclease h activity and then its transfer to the three prime end then similarly he on this and it will start trans it will start here may it will start reverse transcribing this end from three prime to five prime.

So, initially started from five prime to three prime from hundred base prime in here it has a terminal redendent terminal which is removed. So, once it is separated its text here because remember these are direct repeats. So, once you have a see DNA complementary DNA of this it will be complementary to this also, right.

So, it will come here into stick here and then the reverse transcription we go from this end to this end. So, once it has been reverse transcribe from this end to this end, then it will remove all the unnecessary portion of the genetic material for example, this one and then it will complete DNA and then it will become part of the other DNA to the integrate

with it and once it has integrated with host chromosome DNA. Now it is in a provirus state. So, now, it will just wait for this part of the DNA to become to be transcribed and once it is transcribe it will have its m RNA and then the m RNA will make the protein requires to make the viral.

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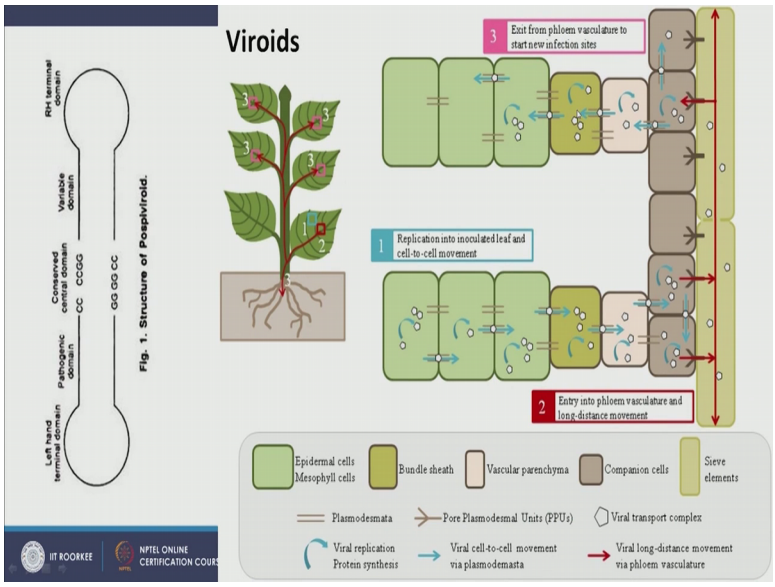

Once promised let us talk about viroids. So, viroids as I mentioned earlier these are single stranded RNA, but they differ from retrovirus and cells that to do not have anything else. So, basically it is the single stranded RNA that moving from one host cell to another host cell and infecting them mineral in return now the obvious question is we must ask is if it is only single stranded RNA how is it stable how does it survive the environment.

So, as I mentioned earlier that RNA have short lives in single stranded RNA will have shorter like than double standard RNA a single stranded DNA shorter, then double standard RNA and both DNA and RNA in the external environment such as the air water fomite they dont last very long that is why they need the capsen capseniers the protein coat to allow them to survive the harsh environment because in the cell its human it is wet and stuff the perfect environment for them to undergo metabolic process was the out in the environment they have to live like a particle devoid of all necessary ingredients for life.

So, how does a single stranded RNA was survive the it. So, happens viroids because of turning them in plants and we notice that they do not survive out on the environment they just transfer from one cell to the neighbouring cell to the neighbouring cell to the neighbouring cell. So, main fraction spreads side by side and that is.

So, that if it were a bond, but you know in plants by the time the infection is operated quite bad now it is single standard, but does not mean that it all that nucleotides are exposed and waiting for anything to come in make hydrogen bonds with its a GTC what it does is it has a conserved central domain. So, within your single stranded RNA they will be conserved domain which are complementary to each other. So, they fold in a way. So, that the conserved domain are complementary. So, that it looks something like this. So, here I have possible viral.

So, in this viral particles there is a central conserved domain in C C C C C G G perfect complement G G G C C. Now notice this is c g rich it is rich in cytosine in morning and if you remember from the first few classes cytosine and gone in have triple bonds between them. So, they are strong bonds. So, this they create very good reason for this viroid to stay in this over left phone. So, when it is makes over left phone we have the conserved centre domain with make sure that none of the nucleotides susceptible to anything coming and making hydrogen bonds with the destroying the integrity of the viroid. So, this is a job of conserved central domain and then we have the variable domain which varies from. So, one viroid to another one type of viroid to another then you have the irsterminalia that right hand terminal in the left hand terminal and you have a typical pathogenic domain which in coats for protein that call make it pathogenic ok.

So, let us see how it works in the cell first it will now let us say if this is a plant and this is a healthy plant, but a inoculate it with a particular viroid and when I have a inoculated into the viroid what will do first epidermis in replicate in the nucleated go from cell to cell. So, it is as I mention from one cell to another it never exposing itself to the environment because the environment is hostile outside the cell, but within the cell if just defined. So, note here on this viroids is folded single stranded RNA it will move from one cell to another an each time it makes multiple copies.

So, each time, it make multiple copies three copies and more copies and they keep moving the second is that when the at least the cell leaf and it enters the phloem

musculatures know it can move through the vascular system of the plants and then once it is move through the. So, this is bundle sheath and vascular parenchyma and then it goes to the companion cells and now with seconds into companion cell with him go to the same element and then it can move want to. So, once it gone same element it can move very easily now these cells are longer and they are very good for transportation and then here look they can go other leaf.

So, they can have from one leaf infection spread to other leaf now the beauty is note here is a this is plasmodesmata and this showing the viral replication. So, viruses making more viral copy one made three and then more and then more and then more at every step. So, it is replicating everywhere accept in the sieve element and this is long distance movement is a short distance movement, but this is very nice and important to know how viroid moves from one cell to cell until the entire tree is destroyed and tree or a plant has lot of plant cells and never exposes its stuff to the environment ok.

Now, look what about the other damages than viroid can do.

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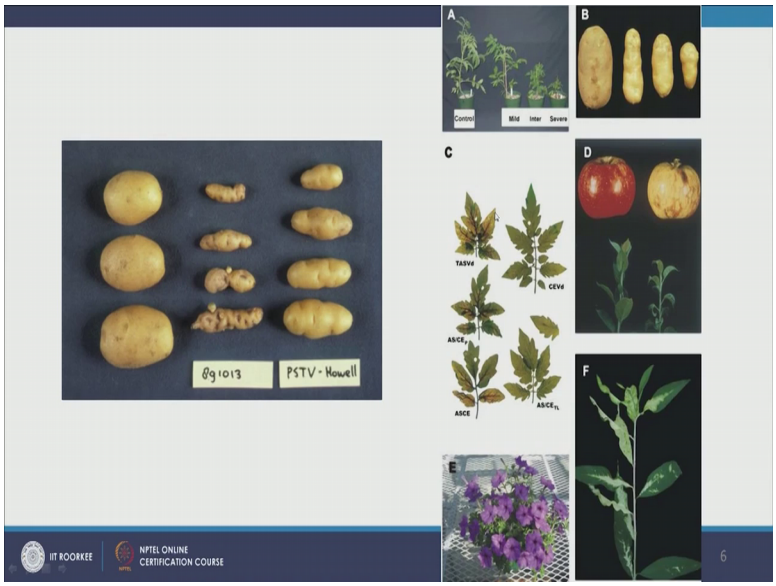

If you look very carefully in this picture these are healthy potatoes and these are potatoes infected by this voiroid; these are potatoes infected by these viroid grown under otherwise very similar conditions and you notice how they can affect your agriculture productivity. So, this is not just a plant disease problem, but it becomes very soon and economic problem and then the social problem and then a public health problem because

when people do not have food to eat, then we have then just susceptible to other diseases similarly if you look at these tomato plants this is the control and then this has mild infection intermediate infection and very severe infection.

So, it might cause blight of (Refer Time: 14:03) that we can only fear a healthy apple; apple infected by viroid and even the plants is not just the size of the plants or colour of the fruits or the quality of the fruits and vegetables that diminishes, but also the morphology of the plant changes the colour of the plant changes ok.

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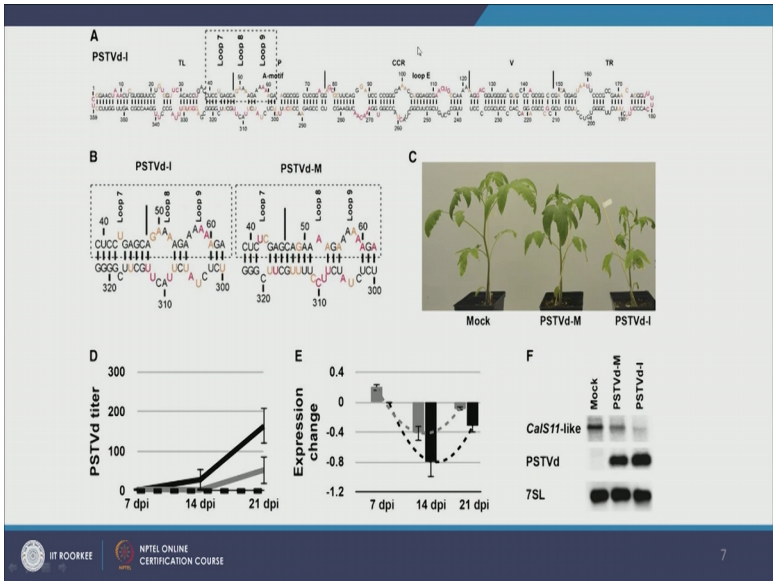

So, this is a very nice study that was recently published about viroids and they actually would; now they using (Refer Time: 14:32) a tools you can actually sequence of voiroid and look here not only did this sequence the viroids, but they got also see how the viroid SS RNA single stranded RNA force on to its and in the G C rich regions complementary G C rich regions. So, if you look here that perfect complement here or the perfect complements are in black and the others synondroids. So, you have domains that are loop they are not very they are not complementary to each other.

So, this stay loops, but you have lot of complementary region and more there it is very G series region if you go through them very nicely and look and they identified the pathogenic zones on in this region which zones are pathogenic and they also notice that when we changed when you cause changes in this pathogenic zones the health of the plant changes decreases or increases very good study and this is an example for you on

how you can become future researcher and look after a plant health our agriculture output the health of even trees.

So, that are forests in our green tree maintain law and look here you have you must be familiar with sequencing techniques by now the once that I talked earlier. So, the sequence is not only tell us who is present in terms of taxonomy they not only tell us what it is doing which we get when we do functional invitation of our sequences, but they can also help us understand the molecular biological background of a phenomena for example, we know that this is a viroid, it causes diseases in a tomato plants, but we do not know what part of it are pathogenic what elements in this viroid pathogenic we dont know the structure of the pathogen of this viroid we do not know because if you know the structure we know the loops that are pathogenic.

We can tackle them we can make drugs that will interfere with these loops particularly and then they will not allowed these viroid to affect because they single stranded r n a. So, they will not allowed into translate and this affect the health of the plant. So, in this particular study the user sequencing techniques and then they use some ensilica a modelling ensilica tools that allow them to fold a single stranded RNA. So, that the perfect complements met each other and look how beautifully starts from here from one it is 360.

Some baseperent long viroid and then it full shape perfectly in near 180 and then it comes at compliment. So, it is a very very is a fantastically smart approach of protecting in still being single stranded RNA the beauty of single stranded RNA that you get directly translated, you do not have to waste plant recourses to undergo the transcription process, you do not have to become integrate into that holds DNA and then wait for DNA to translate transcribe your part of the genome, alright.

So, now we move on to prion. So, we talked about virus we talked about viroid and now let us talk about prion. So, on one extreme we have pathogenic bacteria these are full sieve functional viable bacterial cells that can infect animals it can infect animals such as us and cause diseases in us then we talked about viruses which are neither alive not there, but somewhere in there depending on the context that inside the host they can replicate. So, they might be alive, but it outside not alive.

But now, we are moving on and viruses you know; they do not have functional proteins they only have protein coats that keep them safe in the external environment then you talked about viroids which are devoid of everything except single stranded RNA and viroids are the smallest pathogens at times.

So, 600 if you look here again, just 360 nucleotides 360 based that is good tiny tiniest passages but now, prions are very interesting that they do not have any genetic material that just proteins just proteins that are naturally and beauty while, they do not need any genetic material is because the proteins and the gene for the protein is already found inside the animal typically in the neurones typically inside the brain this is the reason why we have mad cow disease and the prion disease that eats of the brain of human beings also this is a picture.

How a prion infest the brain would look.

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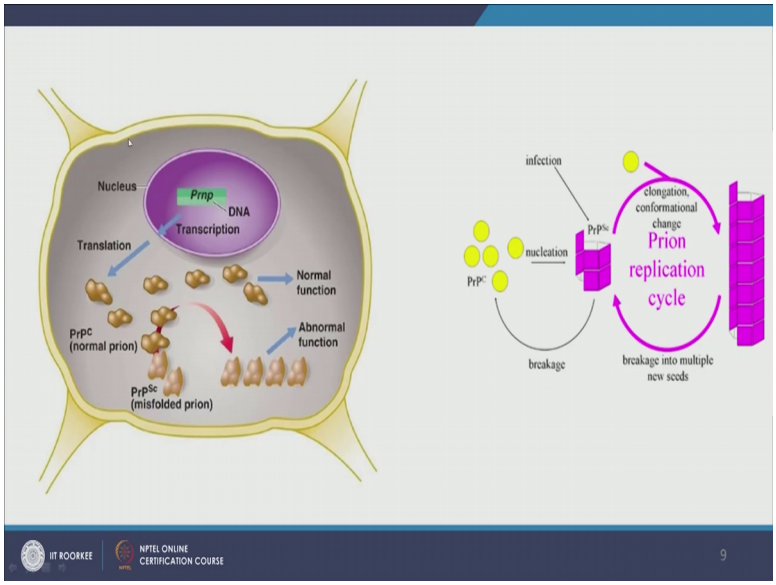

So, how does prion work let us look here? So, as I mentioned the eukaryotic cells specially the nuclear cells and you can tell this is nuclear cell because your absence extending out here. So, these are the tails of here neurones and they already in the nucleus this is nucleus the already have a gene called P r n p gene. So, P r n p gene makes the P r p c protein. So, P r n p gene is prion protein gene prion protein gene it makes P r p c protein which is a normal prion protein and it does its job this is nothing wrong in it, but if it undergoes a miss folding it gets miss folded then this P r p c

becomes P r p s e and this miss folded protein has abnormal function now you have P f p r p c here. So, this is healthy protein.

It undergoes nucleation it becomes on miss folded protein P r p s e and now what it can do it; it makes other prion proteins undergo elongation conformational change that more prion breaks into and so, these. So, these protein; now become like this and this stack on to each other and then it breaks into makes new seeds. So, on miss folded protein big deal, but no this miss folded protein test at the damaging the brain cells and starts causing really bad disease now this is call pathogen even though it is and a seems like miss folding protein problem because the miss folded protein now activates and make other well folded protein in 2 miss folded protein. So, if you remember in the last lecture.

When I was talking about classifying things into living and nonliving being living beings the criteria that most scientist agree on its they can replicate. So, we a cell can replicate its alive a virus can replicate inside a host maybe alive may not be alive a protein can activate on its own now we are talking about that pathogen even though it is not alive because it can survive its own its not it does not even have a genetic material, but it causes other prion proteins to become sick miss folded protein.

So, in this sense it is replicable and it is alive; now prion disease is a major disease in dairy industry when it causes the mad cow disease it is also out of affect human being though it not very common for human being, but very common for dairy now they will just study recently that is; so, how consumption of wheat affected by prion can be a source of infection.

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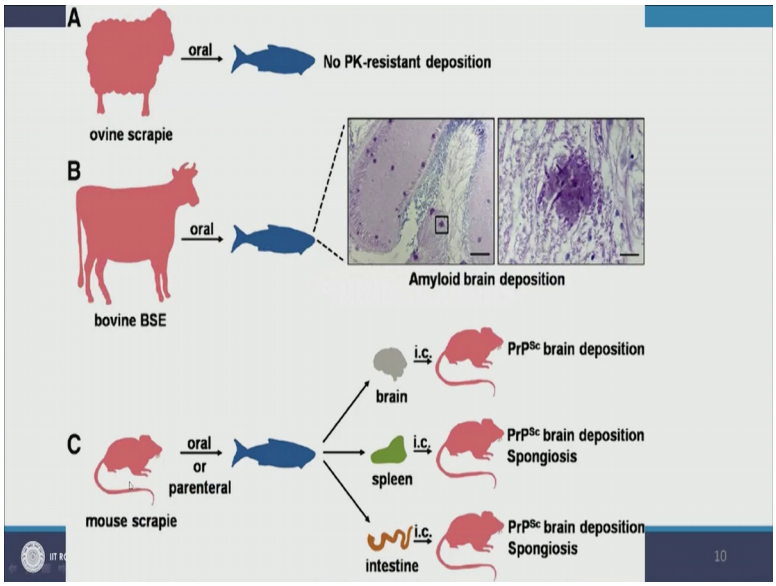

So, what they did was that they took difference animals that world affected by prion in the fed meet of that animal 2 fishes and the notice when they fed it from the sheep there was no P k resistant deposition, but when they fed it from bovine that infected by prion; they notice changes happening in the brain same thing from scrapie the mouse they notice that brains spleen intestine all of them had been issues you must have noticed that miss folded protein is called P r p s c and the reason is because the normal protein P r p c when it was miss folded it was first detected in the scrapie mouse.

So, S c stands for scraping stand protein is scrapie (Refer Time: 22:27) ok.

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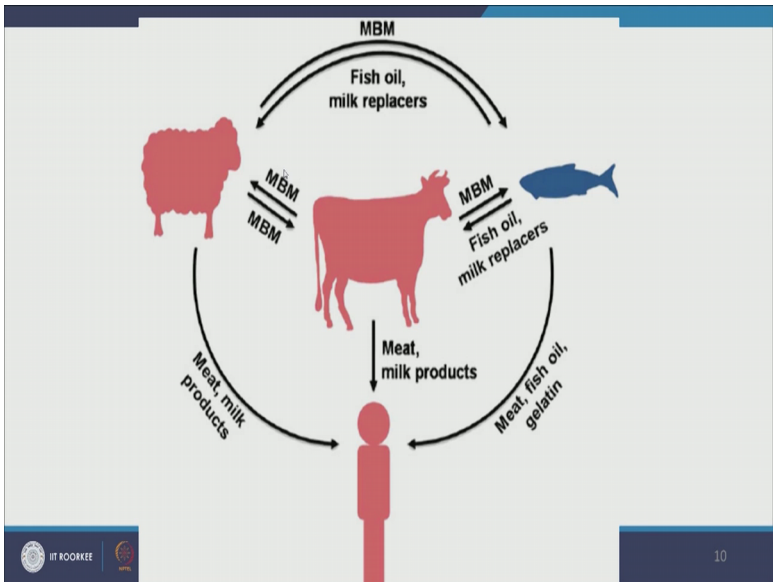

So, after doing the study with us saw this fed prion infected animals meet different animals in saw were then infection undergoes look here in the last one they had they fed the mouse scrapie time infected scrapies need to fish and then the different parts of fish was fed to the mouse and healthy mouse again and they notice that the prion miss folded prion protein was brain whether the mouse at brain part spleen part intestine parts. So, to prion can know now. Now this is another evidence that suggested prion actually pathogen is not just a case of miss folded protein because not only can it replicate within the cell, but it can go from one sick animals another.

If you go back to one of the first thing I thought was cost pos postulates on identifying a pathogen and 2 main criteria was it should be able to replicate it should be able to be isolated from sick animals and then you should be able to be maintained a grown in the lab and then it should be able to infect healthy animals in this is exactly what the observe. So, prion is the definitely a pathogen.

So, when they were done with their study they have this tentative proposed cycle of how can transfer well you have mad cow mad cow has a cow infected by prion and fishes eated it; fish if fishes infected cow can get infected if cow infected eats some body parts are used for making fish meal the fish can get infected human bite assume fish parts it meat fish oil gelatin in humans can get infected even through the meat of the cow the

buffalo and the milk products similarly the cow and sheep they interact some they interact with each other then might spread the disease.

And then the sheep meat milk production can infect you human being same thing if sheep and fish interact like perfect sheep of fed fish for example, right or milk replacer for example, in many of the industry forms the sheep baby and cow baby do not get to suckle their mother because their milk is directly taken and she siphoned off for human consumption. So, the young links specially the female because the male are killed off they females they are given milk replacer. So, the most of them come from fishes this herbivorous eat carnivorous food in they that is how the prion can spread from can be transmitted from one body to another. So, this is about prion.

So, we have talked about virus we have talked about virals that we have talked about prion. Now these 2 days, I want to give an example of a viral disease that is currently rapid drumpent India as a record this lectures august twenty seventeen. So, earlier these years H1N1 had being had spread in India and if you look until May 7-17th.

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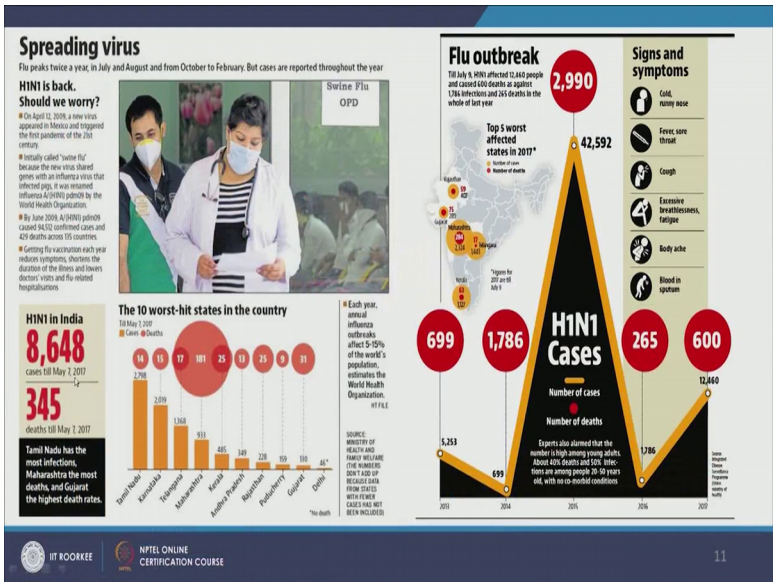

We had some 8000 H1N1 cases in India, 345 deaths in India. So, this is and 2017 and I am recording this picture, this is a flash back into 2009 when the swine flu H1N1 flu spread in North America and South America and it cost up to 94,000 cases, there some 500 deaths and may people encourage people to get flu vaccines very year. Now this is a

reminiscent of that because India's arm infection rate is very high and I personally do not know the flu that I have right now if it H1N1 or not, but if you notice that.

We have noticed that especially in the Deccan; the southern part of the country, we have quite high range of number of cases and number of death; for example, in Maharashtra we have us enormous amount of 181 deaths of 993 cases. This is a very high mortality rate; this is 5 percent. So, one fifth of the people who were sick confirmed cases of H1N1 the high that is 20 percent mortality rate. So, now, another thing to notice that as virus H1N1 transmits from one person to another transport from one part of the country to another one country to another country, it undergoes evolution is. So, its mortality rate might also change is also possible that the mortality rate is different 45 states because healthcare is different also known as these are confirmed cases.

So, many unconfirmed cases that never go to may seek medical attention like my own case right now they are not accounted for this in this. Now let us look at this info graphics here it is July 9. So, this is little bit more recent some 12,460 people were affected by H1N1, cost 600 deaths against 1800 infections and 265 deaths last year. So, this year, we have nearly 8 times worse, already we have just above half the year gone in newly 8 times worse H1 N1 of bread in the worst affected states of definitely Maharashtra with their extremely high mortality rate, know, we see it is less than 20 percent, but nearly 30 percent; Telangana, Kerala, Rajasthana and Gujarat, if you do not know how in next few months the diseases spread across; I believe is already spreading in NCR in Delhi, we do not know, but if you look here.

The worst; we had a of in past few years in 2013, they were some 5000 cases of confirm cases of H1N1 and from 700 deaths in 2014 the we have lesser number of H1N1 cases, but we had higher; sorry in yeah high mortality in 2015, we had some 42,000 cases confirm cases of H1N1 which is a big number and some 3000 people died in 2016, they had drop. So, only 1700 cases of confirmed cases of H1N1, but now is increasing again we do not know when we are going to have another major outbreak of H1N1 an even that sounds like a public health problem, but this is also a environmental problems.

For example, we do not know still H1N1 virus how long it possess on four miles on the surfaces. So, this is environment issue how case should we maintain our environment how often should we wash our hands, alright and how does H1N1 work; this is a very

unique case because in H1N1 virus especially a H1N1 virus which is what we are facing right now it is the virus has mutated and involved in three different species in the bird in the humans in the pig.

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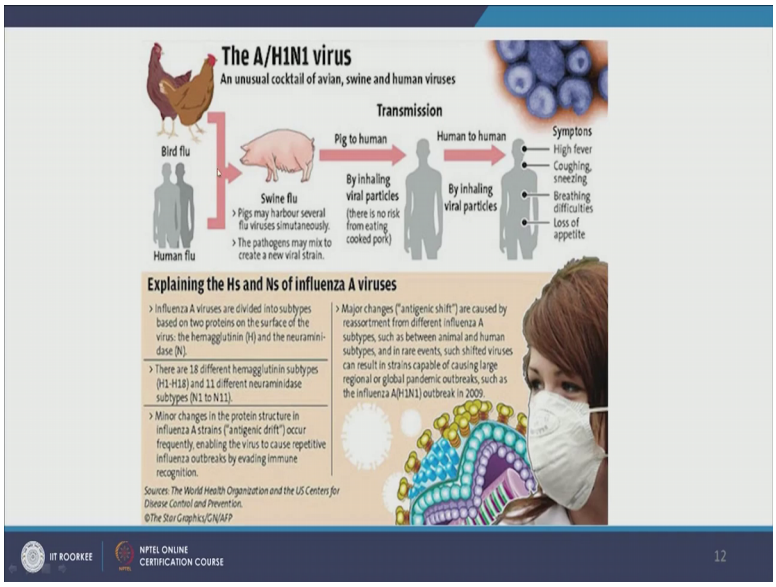

So, the first the bird flu and human flu combined genomes the virus and they came up and they infected in pigs in the make world here and then it infected human and no we have a really bad H1N1 issue, alright, dear students, this is all for today and in this is all for the virus in the next class we will take up the first environmental microbiology problem in I will walk with through the problem how we apply the microbiological tools I will understanding and how we solve it.

Thank you.