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REVIEW ARTICLE

NEXT GENERATION SEQUENCING AND MICROBIAL COMMUNITY ASSOCIATED WITH EUKARYOTES INCLUDING PARASITIC HELIMINTHS: A REVIEW ARTICLE.

[*]Saeed El-Ashram[1,2], Ibrahim Al Nasr[3,4], Rashid mehmood[5,6], Min Hu[7], Li He[7], *Xun Suo[1]

1. National Animal Protozoa Laboratory, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China.
2. Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt.
3. College of Science and Arts in Unaizah, Qassim University, Unaizah, Saudi Arabia.
4. College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass 51921, Saudi Arabia.
5. College of information science and technology, Beijing normal university, Beijing, china.
6. Department of Computer Science and Information Technology, University of Management Sciences and Information Technology, Kotli Azad Kashmir, 11100, Pakistan.
7. State Key Laboratory of Agricultural Microbiology, Key Laboratory of Development of Veterinary Products, Ministry of Agriculture, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei,China.

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Abstract

Molecular techniques, such as 16S rRNA sequencing and whole microbial genome sequencing, have revolutionized the standard microbiological practice by disclosing the remarkable diversity, composition and identity of bacterial communities associated with eukaryotes. Here, we outline the development from the single-gene analysis in an ecosystem to the compounded genetic information of the entire ecosystem employing high-throughput sequencing technologies. We also provide the microbial communities associated with eukaryotic organisms, including some parasitic helminths. We hope that the information provided here will be useful to widen our understanding of the techniques in most reasonably equipped molecular biology laboratories, and bacteria associated with eukaryotes, involving parasitic heliminths.

Co-Corresponding Authors:- Saeed El-Ashram and Xun Suo.
Address:- National Animal Protozoa Laboratory, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China.

The necessities of microbial world:-

Prokaryotes represent the largest proportion of life forms on earth, which comprises 10^6 to 10^8 different genotypes. The largest reservoir for carbon (350–550 Petagrams), nitrogen (85–130 Pg) and phosphorous (9–14 Pg) is the prokaryotic organisms that inhabit our planet [1]. These microorganisms are responsible for most of the chemical cycles on earth, which are essential for our existence. In addition, microorganisms serve human beings by maintaining our health, fermenting food and producing drugs [2]. Most of these microorganisms live in communities; many of those communities are complex with high magnitude of diversity with thousands of interacting members [3] where they will compete for basic needs like space, air, etc. For the better understanding of life, it is essential to understand the diversity of these microorganisms in the community. Most of the research on microorganisms is based on culturing organisms in the laboratory. Major difficulty encountered by researchers in the field of microbial study was, how to study those organisms which do not grow under standard culturing conditions [4]. The term metagenomics was first coined by Handelsman in 1998, for habitat based investigation of mixed microbial population at the DNA level. Metagenomics provides a culture–independent way to access unculturable microorganisms and is now possible to study the genome of all those organisms. Thus metagenomics revolutionized the field of microbiology which offers a window to understand previously unknown and uncultivable microorganisms. Life on earth was flourished due to a transition from the anaerobic to aerobic forms of photosynthetic bacteria. Due to this transition oxygen began to accumulate in the atmosphere until it was sufficient to support the life of aerobic organisms. Once oxygen concentration reached at a very high concentration, oxygen molecule began to collide and produced ozone. Later this ozone gas accumulated in the stratosphere and protected the life forms on earth from ultraviolet light [5]. Yet another group of microorganisms evolved are nitrogen fixers. These bacteria could break triple bonded nitrogen molecule and fix atmospheric nitrogen for the usage of terrestrial living beings. Human health is under constant check by human microbiome such as gut microflora, when the balance of gut microbial community is compromised, many diseases like colon cancer, inflammatory bowel disease, obesity and diabetes may occur. All these microbes coevolved with the human species, produces an intertwined web of dependency and communication. A large proportion of the drugs available today are synthesized from bacteria and fungi. The discovery of antibiotics has transformed human existence by providing an outstanding way for the treatment of infectious diseases. In addition microorganisms play an important role in providing industrial enzymes and polymers, cleaning up toxic waste products and can be employed in the process of fermentation etc. Tabulated below are the differences between cultivable and uncultivable microbes [6]. In the late 1970s, the microbial genome study began with the sequencing of bacteriophage genomes MS2 RNA [7] and QX174 [8]. *Haemophilus influenza* genome was sequenced in 1995 [9].

History and technology evolution:-

There are many examples in the scientific literature of bacterial groups co-existing in the animal and human body. Their composition and activities are thought to be closely involved in shaping our health and have been examined over several years. The classical approach to explore the animal and human microbial community is relied on culturing and isolating a single microorganism, and identifying its physiological, biochemical, ecology, life history and serum reaction index characteristics. Nevertheless, a large proportion of the gut microbiota is uncultured microbes, and it would be logistically laborious to grow the diverse species that can be cultured. With the development of molecular biology and the extensive application of modern technologies, many new genomic approaches have emerged during the past decade for the animal and human microbe investigations. These approaches involve terminal restriction fragment length polymorphism (T-RFLP), PCR based denaturing gradient gel electrophoresis (DGGE), automated ribosomal intergenic spacer analysis, fluorescence in situ hybridization (FISH), and microarray.

Sanger sequencing and next generation sequencing (or high-throughput sequencing):-

Sequencing technology has become the common process in researching the animal microbial community, and metagenomic sequencing has firmly transferred from conventional Sanger sequencing technology to be next-generation sequencing (NGS). The disadvantages of Sanger sequencing are the laborious cloning process, the cloning bias and the expensive cost of a single giga base of data generated. The most conspicuous source of this bias, which produces a low read coverage in these regions, is adenine-thymine (AT) richness. NGS, which eliminate the cloning bias, has entirely altered sequencing efficiency, allowing high-throughput analysis of complex microbiota via the capture of short DNA sequence (amplicons or random fragments) in which a myriad of samples may be multiplexed employing short DNA sequence “bar codes” [10]. This provides adequate sequencing depth in each sample to characterize the top 99.99% of the microbiota, permitting the unravelled micro-organism discovery with no prior knowledge of their sequences. In addition to analyzing the microbial genomics, NGS is also

appropriate for transcriptome sequencing, which determines mRNA quantitatively, enabling new insights into genome expression and how it may be altered in healthy and diseased individuals. The NGS technology holds promise for a contemporary understanding of infectious disease and for diseases not previously known to have a microbial element.

The different sequencing strategies:-

In recent years, various types of sequencing strategies have been applied successfully in a wide range of metagenomic projects that have enormously revolutionized our knowledge of the microbial communities living in and on animal and human bodies. A large and growing body of literature on the microbial community is relied primarily on three kinds of sequencing strategies: amplicon sequencing, shotgun sequencing and transcriptome sequencing, whose cardinal goals are to study microbial communities *in vivo*. The most common application of amplicon sequencing with NGS technology in the microbiome is 16S rRNA gene sequencing, which is usually employed to assess the bacterial general composition. Metagenome shotgun sequencing provides more intricate information of the microbiome, including gene content and its potential functions. Finally, the active members of the microbial community and its functionality can be investigated by transcriptome sequencing.

Amplicon sequencing:-

Complex microbial communities, such as the animal and human gastrointestinal tract (GIT) microbiome, are presently receiving increasing interest, owing basically to technological advances in culture independent approaches in recent years. Nevertheless, in surveys of tremendously diverse ecosystems, the size of clone libraries (typically 100–500 clones each) only allows for identification of the community members that are present in huge abundance [11; 12; 13; 14; 15; 16]. In addition to failing to detect rare members of the ecosystem, these relatively small data sets provide inaccurate estimates when used for computing species richness within an ecosystem. Regardless of the approach used to estimate species richness, the estimates acquired are highly dependent on sample size, and smaller data sets usually result in the miscalculation of species richness [17; 18]. However, it was not until the end of the 1970's that ribosomal ribonucleic acid (rRNA) sequences were observed to provide a key to prokaryotic phylogeny. The pioneering research and view of [19; 20] was approved and taken up by others [21; 22]. The rRNA molecule is universally distributed, functionally homologous across all prokaryotes and has regions that are conserved-thus permitting sequences to be aligned for comparison and thereby fulfilling the criteria of an exceptional chronometer [19]. In contrast to physiological and phenotypic traits, the genetic material is not impacted by culture conditions. The number of differences in nucleotide sequences in a specific gene that are counted, the greater the difference between genes, the more evolutionary separated are the two organisms. The evolutionary distances, taken as a fraction of sequence differences between pairs in a collection of sequences, is employed to assemble phylogenetic trees [23]. Because of the slow rate of evolutionary change of small-subunit rRNA gene sequences, along with ease of extraction and manipulation, they have become the molecule of choice, and form the "gold standard" in the creation of phylogenetic classification strategies. 16S rRNA gene sequence analysis has not only facilitated new insights into the phylogenetic interrelationships of microorganisms but has provided molecular systematists with an immensely powerful means for describing new diversity within any given microbiological environment. The molecular approach has had major repercussions not only in taxonomy but also, for example, microbial ecology. Studies by [22] have demonstrated that microorganisms could be identified directly in their habitats through a combination of rRNA gene cloning and sequencing. The 16S rRNA gene is a constituent of the 30S small subunit of prokaryotic ribosomes. It is approximately 1.5 kb (or 1500 nucleotides) in length (Fig. 19) and is employed for phylogenetic studies [24] as it is extremely conserved between different bacterial and archaeal species [25].

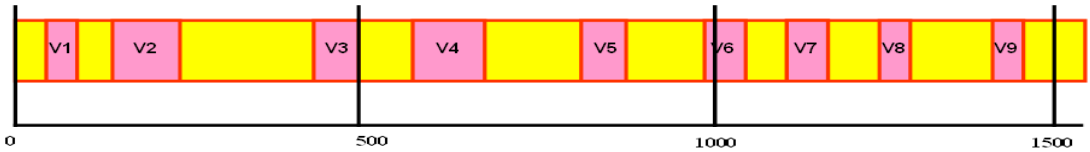
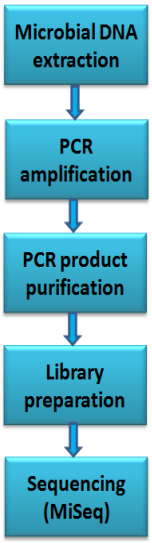


Figure 19:- Nearly 1.5 kb 16S rRNA gene of *E. coli* illustrating the nine variable regions.

The 16S rRNA gene consists of conserved and variable regions (Fig. 18). The variable regions, which are nine different variable 16S rRNA gene regions being flanked by conserved sites in most bacteria [26], allow discrimination between different microorganisms. 16S rRNA gene methods rely on the PCR (polymerase chain reaction) using ‘universal’ primers targeted at the conserved regions and designed to amplify as wide a range of

different microorganisms as possible [27; 28].. As a result, 16S rRNA gene sequencing has become ubiquitous in microbiology and microbial ecology as an expeditious and precise alternative to phenotypic bacterial identification methods [29]. Currently, the sequencing of 16S rRNA gene variable regions is the most common application of amplicon sequencing on NGS platforms (Fig. 20).

It provides a quantitative description of the bacterial community present in a complex biological mixture, allowing examination of entire communities and the identities of their constituent members. Phylogenetic mapping of rRNA variation was first used to create the three domain of life [30]. The gene conserved nature was subsequently exploited to develop more rapid methods for determining relationships between organisms directly from environmental DNA and RNA extracts [27; 31]. The coupling of 16S rRNA PCR with next-generation sequencing enables the study of a wide range of samples at low cost [32]. There are three benchtop high-throughput sequencing instruments, which are the 454 GS Junior (Roche), MiSeq (Illumina), and Ion Torrent PGM (Life Technologies). They are laser-printer sized and offer modest set-up and running costs. The MiSeq had the highest throughput per run (1.6 Gb/ run, 60 Mb/h) and lowest error rates. The 454 GS Junior generated the longest reads (up to 600 bases) and most contiguous assemblies but had the lowest throughput (70 Mb/run, 9 Mb/h). The Ion Torrent PGM, which runs in 100-bp mode, had the highest throughput (80–100 Mb/h). In contrast to the MiSeq, the Ion Torrent PGM and 454 GS Junior both produced homopolymer-associated indel errors (1.5 and 0.38 errors per 100 bases, respectively) [33]. 16S rRNA data analysis depends on related sequence clustering at a specific level of identity and counting the representative number of each cluster. Similar sequence clusters are known as operational taxonomic units (OTUs). A level of 95% sequence identity is frequently chosen as being representative of a genus and 97% for a species when using partial 16S rRNA gene sequences [34]. Identification accuracy is reliant on the selected reference database. Curated databases, for example the Ribosomal Database Project [35], GreenGenes [36] and SILVA [37], where quality assessment and alignment of MiSeq sequences are manually optimized, are substantial for optimal phylogenetic analysis. There are two common analysis pipelines employing for analyzing 16S rRNA gene sequence data. These are: QIIME [38] and Mothur [39]. Figure 1 and Table 1 present the summary statistic of the community structure, and the commonly employed ecological and NGS term explanation in the microbial community research field. The commonly employed ecological and NGS term explanation in the microbiota research field is illustrated in Figure 21 and Table 3.



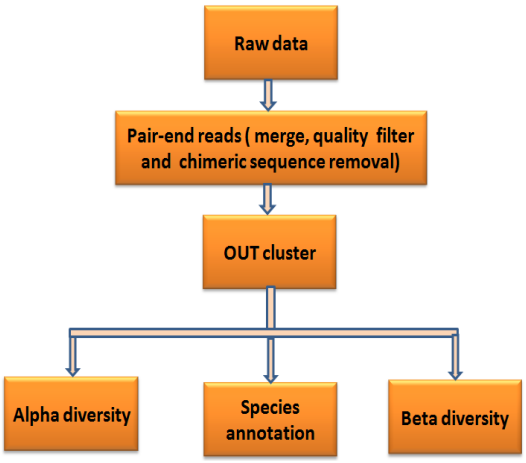


Figure 20:- Flow chart summarizing the 16S ribosomal RNA (rRNA) NGS

Table 3:- The commonly employed ecological and NGS term explanation in the microbiota research field.

Term	Explanation	Reference
Operational taxonomic units (OTUs)	Clusters of 16S/18S small subunit (SSU) rRNA gene similarity, are used as theory-agnostic approximations of microbial taxa	[40]
Microbiome	It literally means small biome, the ecosystem comprising all microorganisms in a particular environment together with their genes and environmental interactions.	[41]
Microbiota or microbial community	The assemblage of microorganisms themselves and can include bacteria, archaea, viruses, phage, fungi and other microbial eukarya.	[41]
Bacterial community	The composition of bacteria living within a particular part of the body.	[42]
Microflora	The community of microorganisms, including bacteria, fungi and algae, which live in a special habitat or in or on another living organism.	[43]
Flora	Referring specifically to plants, rather than microbes	[41]
Bar code	For processing a large number of samples with multiplex sequencing on a high-throughput instrument, individual "barcode" sequences are added to each sample so they can be distinguished and sorted during data analysis.	[41]
Evenness	A measure of the skew in abundance of community members. Is there one dominant organism or are all evenly represented?	[41]
Richness	The number of different types of organism present.	[41]
Diversity	A combination of richness and evenness can be considered to be a summary statistic for community structure.	[41]
Simpson index	A common diversity index indicating the probability that two individuals taken at random from a population are the same. Often presented as the inverse so that increasing diversity is mirrored by an increasing index value.	[41]
Shannon index	Alternatively, Shannon entropy-another common diversity index that quantifies the uncertainty of predicting the next individual taken from a sample.	[41]
Alpha diversity	Within sample diversity.	[44]
Beta diversity	Between sample diversity.	[44]

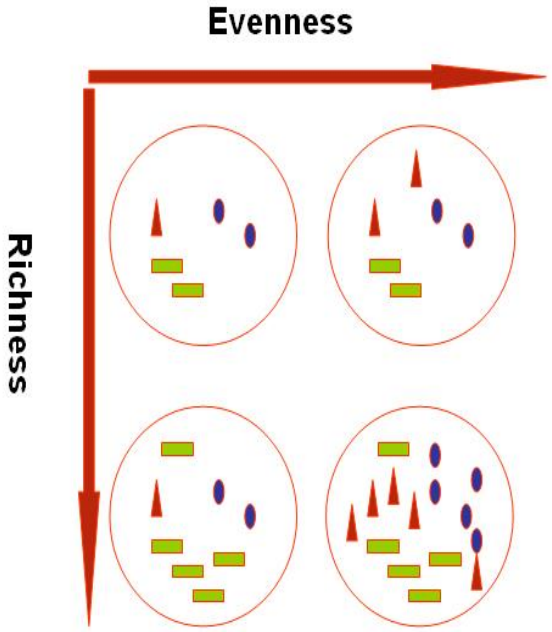


Figure 21:- A diagram describing species richness and evenness and how they characterize the community composition. Each shape represents an individual and the color and nature of the shape represents a different type of organism. Increased numbers of diverse types of organism is characterized as augmented species richness. The community is described as even, when no one organism is dominant.

Factors affecting gastrointestinal tract (GIT) microbiota:-

The ruminant gut microbiota is crucial in shaping several of its host's functional attributes. The bovine rumen microbiota is necessary for the proper physiological development of the rumen and for the animal's ability to digest and convert plant mass into food products, making it highly significant to animals. Factors affecting gastrointestinal tract (GIT) microbiota composition and activity in ruminants are presented in Figure 21. The bacterial profiles of the rumen microflora is known to be highly responsive to changes in diet, age, antibiotic use and health of the host animal and varies according to geographical location, season and feeding regimen (feed and feed additives) [45; 46; 47; 48; 49; 50]. Additionally, it has been reported that the host gut microbiota varies across species and individuals; however, it is relatively constant over time within an individual [51]. However, it has been reported that the detectable bacterial structure in the rumen is remarkably conserved among diverse locations and over time, while the quantity of individual bacterial species may alter diurnally in response to the feeding regimen [29]. The diversity and within-group similarity augmented with age, suggesting a more diverse but homogeneous and specific mature community, compared with the more heterogeneous and less diverse primary community. In addition, the establishment of this microbial population and the alterations occurring with the host's age was observed [52]. The composition of the intestinal microbiota can be modulated as a result of dietary exposure (ovine milk, formulas) as well as of intentional diet supplementations (prebiotics or probiotics). Rumen microbial community composition of cattle, bison, and buffalo (bovines), sheep and goats (caprids), deer (cervids), and alpacas, llamas, and guanacos (camelids), including diverse breeds of domestic cattle, sheep, and goats varies with diet and host [53]. It is well-established that the microbial community structure and composition is affected by diet[54]. The feeding operation is a more essential determinant of the bovine microbiome than is the geographic location of the feedlot [55]. Probiotics/prebiotics have the ability to modulate the balance and activities of the GI microbial ecosystem in ruminants [56]. Host genotype is among the factors that influence the microbial composition of the gut [57; 58]. Some authors studied the similarity degree in the predominant faecal microflora of identical twin pairs, fraternal twin pairs, and unrelated controls; the highest levels of similarity were observed in genetically identical twins [59]. Some authors studied the development of rumen microbial populations in young calves and lambs [60; 61]. The diversity indices of bacterial 16S rDNA and the change in soil bacterial community composition increased under intensive grazing [62]. A growing body of evidence shows that parasitic infections are associated with the alterations of porcine proximal colon microbiota at 21-days of infection with *Trichuris suis* [63], and the changes in the caprine abomasal microbial composition induced by *H. contortus* at 50-days infection [64]. Interactions among helminth parasites, bacterial microbiota, and host immunity have been reviewed in detail elsewhere [65; 66; 67] (Fig. 22).

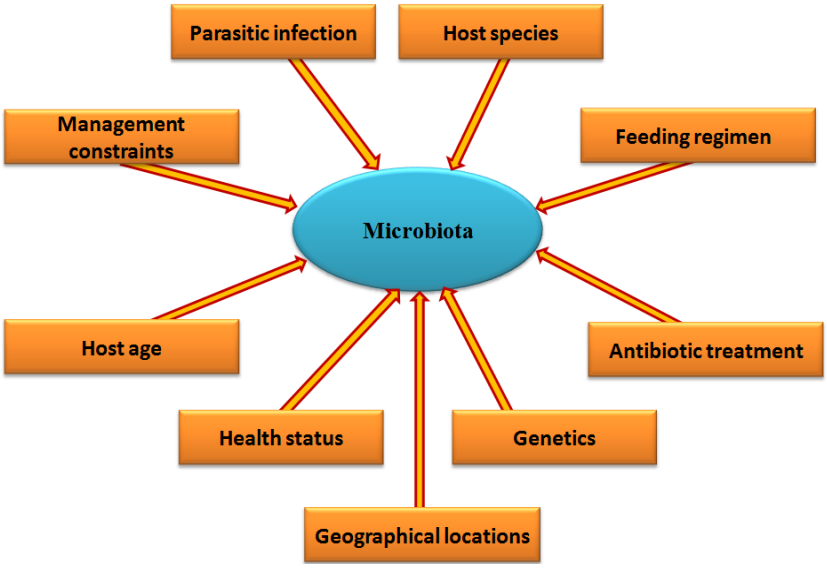


Figure 22:- Factors influencing the development and composition of microbiota in ruminants.

Microbial community associated with eukaryotes:-

A microbiota is an ecological community of symbiotic microorganisms found in and on all multicellular organisms. The synonymous term microbiome literally means small biome, the ecosystem including all microorganisms in a particular environment together with their genes and environmental interactions. The assemblage of microorganisms themselves is referred to as the microbiota or microbial community and can involve bacteria, archaea, viruses, phage, fungi and other microbial eukarya [68; 69]. Microflora can also be existed as a general term in the literature; nevertheless, flora refers particularly to plants, rather than microbes. The animal microbiota consists of microorganisms that exist upon, within or in close proximity to the animal body. Bacteria live in a wide variety of environments and have evolved close relationships with invertebrates, vertebrates, plants, and even with other bacteria [70; 71; 72]. The interaction between host organisms and prokaryotic microbes can range from pathogenic to mutually beneficial [71]. Symbiosis is an intimate interaction between two different biological species [73]. Such associations involve a variety of more specific relationships: parasitism (The symbiont benefits and the host is harmed), commensalism (the symbiont benefits and the host is unaffected), and mutualism (both the host and the symbiont benefit). It can be categorized according to their location in the host as either ectosymbionts (living on the surface) or endosymbionts (residing within host tissues). The latter can further be classified as primary, obligate *P*-endosymbionts (vital for host survival), or secondary, facultative S-symbionts (not necessary for host survival). Some *P*-endosymbionts reside in particular host structures or organs called bacteriomes; however, others are more extensively distributed in host tissues [74; 75]. The symbiosis continuation through host generations depends on symbiont transmission. Horizontally transmitted symbionts are recently taken up from the environment by each host generation, and vertically transmitted symbionts are frequently transferred through the female germ line. Mixed mode of transmission also exist [76]. For horizontally transmitted bacteria, symbiotic life is facultative: a free-living population serves as the inoculum for the symbiosis. Such free-living populations occur in soil [77], marine shallow waters and the deep sea [78]. In some cases the free-living population is restored by symbiont release from the host [79]. Vertical transmission frequently involves no aposymbiotic phase and transmission through the female germ line, even though there may be acquiring during mating. Moreover, there are unusual ways of maternal transmission, as in the stinkbug (*Megacopta punctatissima*), which harbor extracellular symbiotic gammaproteobacteria in the midgut cavity [80].These bacteria are deposited with the eggs on plants in "symbiont capsules", are eaten by the newly developing nymphs and colonize the insect midgut [81].

Symbiotic bacteria:-

A variety of microbes colonize invertebrate and vertebrate mucosal surfaces, including digestive, respiratory, and reproductive tracts [82].

Gut symbionts:-

Invertebrate-microbe associations:-

The life cycle of five invertebrate-microbe interactions has been reviewed by [83]. In each of the symbioses demonstrated below, the animal acquires a specific symbiont (or symbionts), which colonizes the host in a specific location: a) the squid obtains its symbionts (e.g. *Vibrio*) from sea-water populations, which colonize the nascent light organ; b) the nematode brings its symbiont (e.g. *Xenorhabdus-Photorhabdus* and nematode worms) into the insect host (e.g. *Drosophila*), where both proliferate. The bacteria then recolonize the nematodes, which escape from the carcass; c) juvenile leeches obtain symbionts (*Aeromonas, Rikenella* and the leech) after hatching from their cocoon (perhaps from the cocoon itself). They then take up residence in the crop, where they digest their blood meal; d) the tsetse fly (*Sodalis glossinidius* and the tsetse fly) can either pass the symbionts maternally to the eggs or pick up new strains from the environment; e) specific symbionts on the food of the fruit fly colonize and persist in the enteric tract (i.e. *Enterococcus* and the fruit fly) (Fig. 23).

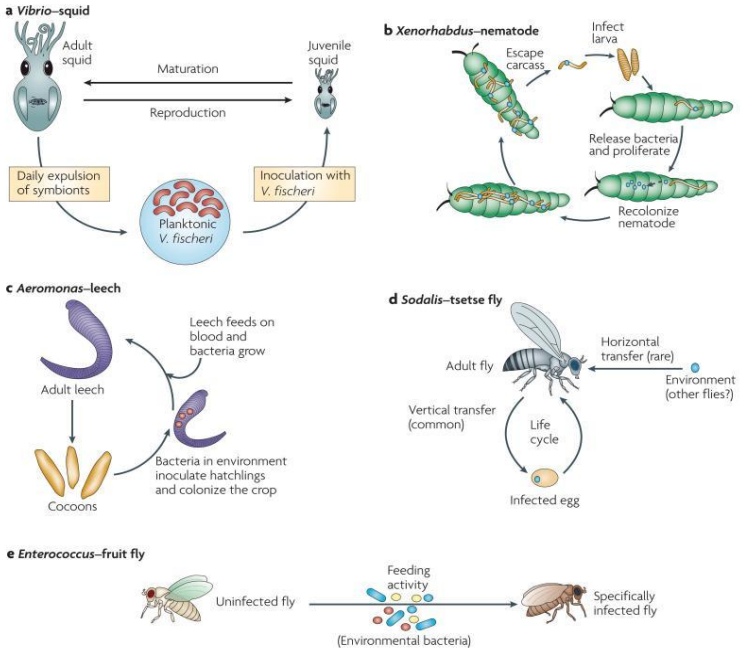


Figure 23:- Simplified life cycles of five symbioses (adapted from [83])

Nematode-microbe associations:-

Little is recognized about the symbiotic gut bacteria of nematodes and their role in host nutrition; however, there are ectosymbiotic microbes of marine nematodes known to supply nutrients to their hosts [84]. While abomasal nematodes are exposed to the abomasal and ruminal microbiota, the nematode gut symbionts may reflect the external microorganisms, which continually pass through their guts.

Rumen-microbe associations:-

The rumen microbes involve many species of bacteria, protozoa, fungi, and archaea, and are dominated by bacteria. These microbes are crucial for the fermentation, digestion, and conversion of indigestible foods and mucus into short-chain fatty acids and microbial protein [85; 86]. The rumen microbiota starts establishing in the lamb rumen soon after birth, before the rumen is functional. In the rumen of lambs, rigorous anaerobic bacteria prevail as early as 2 days of age. Cellulolytic bacteria emerge around the fourth day. Protozoa become established later (2 wk) and their populations have maintained by 4 mo [87]. In field-raised lambs, individual bacteria sequancially colonize the rumen: stric anaerobes prevail at day 2, from day 2 to 10, the strict aerobes and facultative anaerobes are 10 to 100-fold lower than the anaerobes and continue to decrease thereafter; methanogens and cellulolytic bacteria appear by day 3 and reach adult levels by the end of the first week [88]. The prevailing bacterial species in the immature rumen belong to *Bacteroides, Propionibacterium, Clostridium, Propionibacterium, clostridium, Peptostreptococcus and Bifidobacterium* [60], while in adult sheep and goats, the dominant species belong to the genus *Bacteroides* and the phylum *Firmicutes*, including *Clostridium* and *Prevotella* [89; 90]. The rumen bacterial population has been found to be predominantly composed of two phyla: Firmicutes (54%) and Bacteroidetes (40%) [91]. Similarly, it has

been reported that the rumen bacterial sequences are assigned to 19 phyla, with the dominance of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* [92].

Human gut microbes:-
The total population of human gut microbes (10^{14}) exceeds the body somatic and germ cells (10^{13}). The microbial density increases from mouth to the large intestine, with nearly 70% of gut microbes colonizing the colon [1]. The gut is antiseptic at birth, colonization starts immediately after the contact with the mother, and the bacterial number and diversity of the gut flora increases during postnatal development [93]. The microbial population is determnined by the delivery mode (vaginal versus caesarean), diet, age, host genetics (lean versus obese), country of birth (developing world [Pakistan] versus developed world [Sweden]) [94; 95] .

Bacterial enumeration and molecular analysis of human gut samples have shown that the gut microbiota is comprised of approximately 35,000 bacterial species belonging to at least 50 phyla [96]. The gut microbiota are dominated by members of phyla Firmicutes and Bacteroidetes, and there are relatively small proportions of Proteobacteria, Verucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria [97]. The bacterial diversity in the gut lumen is different from the bacterial population attached to the mucus layer, and closely associated with the epithelium [98]. Aside from fermenting indigestible foods and mucus into valuable nutrients for the host, gut microbial community is crucial to the host in diverse ways, including the contribution to the immune system development and homeostasis, epithelial cell proliferation and differentiation, and protection against pathogens [99; 100].

Ecdysozoa microbial community:-
Ecdysozoa is a group of protostome animals, including Arthropoda, and Nematoda [101]. Even though arthropods and nematodes are morphologically different, they have several similar features in their endocrinology and physiology, including molting during development. Bacteria, archaea, and eukaryea, are the gut microbes of insects [102]. Related insect groups tend to share relevant species of symbionts, for example most aphids have primary endosymbionts of the genus *Buchnera* [103] and tsetse flies harbor *Wigglesworthia* in particular cells known as bacteriocytes or mycetocytes [104]. When the gut is simple tube or there is a high-throughput of digesta, the microbial diversity is low [105].

Insect gut microbes:-
The gut bacteria provide essential nutrients for the host, as well as particular enzymes [106], for example cellulase in the termite [107]. The microbial population in the insect hind guts changes with diet [108]. In this context, the bacterial community of wild ground beetles and field-caught *Anopheles stephensi* mosquito larvae are more diversified than those of laboratory-reared beetles and mosquito larvae, respectively [109; 110]. Bacterial community related to *Erwinia herbicola* and *Pantoea agglomerans*, which are ectoparasites of several plants, has been identified in pea aphid excreta cultured on nutritional agar [111]. Obligate and facultative anaerobes are the predominant hindgut microflora of cockroaches and termites [112; 113].

The denaturing gradient gel electrophoresis (DGGE) fingerprinting techniques and visualization with fluorescence *in situ* hybridization (FISH) have been employed to illustrate the microbial diversity associated with insects. Phyla, including Firmicutes and Bacteroidetes are the predominant. Similarly, the gut microbial population identified in ground beetles includes Bacilli, Fusobacteria, Gammaproteobacteria, Alphaproteobacteria, Clostridia, and Bacteroidetes, of which Bacilli and Gammaproteobacteria were dominant in wild and laboratory-raised beetles, respectively [114]. The gut bacterial community of the mosquitoes *A. aegypti* and *A. albopictus* included Actinobacter, Pseudomonas, Asaia, uncultured Gammaproteobacteria [110].

Microbial manipulators of reproduction:-
The best studied endosymbiont microbe that changes the host reproduction in arthropods is *Wolbachia pipientis*, which exploits the host insect to disseminate their progeny [115]. Gut bacteria of fruit fly *Drosophila melanogaster* impacts host mating preferences through pheromone synthesis [116]. Symbiotic *Spiroplasma* protects female *Drosophila hydei* from parasitic wasp attack [117]. Reproductive endosymbionts, including *Wolbachia*, *Rickettsia*, *Arsenophonus*, *Cardinium*, and *Flavobacterium*, which are maternally transmitted, are common among insects. They manipulate host reproduction to facilitate their transmission in diverse ways, such as parthenogenesis induction, feminization, and male killing [118; 119; 120; 121]. *Wolbachia* were early identified microscopically as Rickettsia in insect and arachnid tissues, involving eggs in 1920s [122]. From then on, strains of *Wolbachia pipientis* have been

known as important symbionts of filarial nematodes, while a variety of effects on insect reproduction are associated with the symbiotic *Wolbachia pipientis* strains of insects [123].

Microbial manipulators of host fitness:-
Symbiotic bacteria trigger the host immunity resulting in the regulation of the symbiotic population [124; 125]. Endosymbionts (*Regiella insectocola*) of aphids provide protection to their host against the fungal pathogen *Pandora neoaphidis* [126]. Similarly, the facultative symbionts *Serratia symbiotica* and *Hamiltonella defense* protect the host pea aphid from parasitic infection by *Aphidius ervi* and *Aphidius eadyi* [127; 128]. Symbiotic strategies include competition with the invaders for resources inside the host, synthesis of toxic chemical to assist host defenses, and augmentation of host heat tolerance [129; 130; 131; 132].

Bacteria associated with nematodes:-
Free-living terrestrial nematodes:-
Studies of the interactions of soil nematodes with bacteria have mainly focused on bacteria as a food source [133; 134], model for host-pathogen interactions, and possible nematode biocontrol strategy [135; 136]. Symbiotic bacteria, including Alphaproteobacteria, Gammaproteobacteria dominated in the soil nematode bacterial profiles with absence or very low presence of Actinobacteria [137; 138].

Free-living marine nematodes:-
A number of authors have studied the associations between sulphur-oxidizing, chemoautrophic bacteria with marine nematodes. The nematode parasites provide oxygen and sulphide to the symbiont, which in turn provide food for the host [139; 140]. The cuticle of Stilbonematinae and Desmodoridae harbors sulphur-oxidizing ectosymbionts, which are a source of food for the nematode [84; 86]. The ectosymbiont microbes may be predominated by *Laxus oneistus* and *Robbea* sp. [141].

Entomopathogenic nematodes:-
Entomopathogenic nematodes (EPN) are lethal insect parasitoids, which have been employed effectively for the biological control of lepidopteran, dipteran, and coleopteran pests [142]. The symbiotic bacteria of *Photorhabdus* and *Xenorhabdus* of nematode families Steinernematidae and Heterorhabditidae, respectively are pathogenic in insect hosts and mutualistic in the nematode [143; 144]. These bacteria secrete toxins that are not only toxic to a range of insects, but also to other plant parasitic nematodes, indicating their roles for parasite biocontrol [145].

Mammalian nematodes:-
***Wolbachia* in filarial:-**
Symbiotic *Wolbachia* have been observed in the majority of filarial nematode species, involving the human parasites *Brugia malayi, Onchocerca volvulus, Wuchereria bancrofti* and *Mansonella ozzardi* [146][146][146][145][145][145][356], the dog heart worm *Dirofilaria immitis* [147], and the bovine parasite *Onchocerca ochengi* [148]. They are obligate mutualistic endosymbionts, which are essential for worm embryogenesis, development and adult survival, and essential nutrients to the nematodes [149; 150; 151]. Antibiotic therapy has confirmed beneficial in treating human and animal filarial infections and doxycycline treatment is now extensively recommended in endemic areas [150; 152; 153].

Ascaris suum:-
The facultative anaerobic from the intestine of *Ascaris suum*, including Escherichia, *Enterobacter, Klebsiella, Actinobacter, Cirobacter, Pseudomonas, Aeromonas,* and *Shigella* contribute to the possible serotonin synthesis [154; 155; 156].

Heligmosomoides polygyrus:-
The effects of L_3 larvae and adult worms of *Heligmosomoides polygyrus* on the ileum and caecal bacterial population were investigated, and the adult worm bacterial profiles were similar to those in the infected ileum with the bulk of bacterial species belonging to the phylum Firmicutes. However, the L_3 larval-stage profiles were different from the aforementioned two profiles and composed mainly of phylum Proteobacteria [157].
Trichuris muris:-
The literature has emphasized the necessity of host gut bacteria for the development of GIN parasites. By reducing the bacterial number in the mouse intestine using antibiotic treatment, the number of hatched *T. muris* eggs was

substantially diminished [158]. Several species are known to be affected by the germ-free status of the host include *Nippostrongylus brasiliensis* [159], *H. polygyrus* [160], *Trichinella spirallis* [161].

Haemonchus contortus:-

Few studies designed to identify bacteria naturally associated with GIN parasites of sheep. L_3 obtained by i*n vitro* culture of faeces collected from animals infected with *O. ostertagi*, *Cooperia onchophora*, and *H. contortus* was exploited to identify the bacterial profiles associated with these parasites. The bacterial species identified *Sphingobacterium multivorum* (opportunistic pathogen) and *Streptococcus macacae* (commensal bacterium). The fundamental focus of previous studies on the bacterial interactions with *H. contortus* has not been on symbiotic bacteria, but rather on potential pathogens, such as the soil bacterium *Bacillus thuringiensis*, which could be exploited to control the GIN parasites.The toxic *B. thuringiensis* are commonly employed in the insect biocontrol [162; 163; 164]. More recent attention has focused on the establishment of the molecular identity of abomasal bacteria associated with genetic resistance and susceptibility to *H. contortus* infection in sheep. The observed bacterial phyla with marked differences between resistant or susceptible sheep were Firmicutes (61.4% and 37.2%, respectively), Proteobacteria (10.2% and 37.2%, respectively), Bacteroidetes (12.8% and 5.8%, respectively), and unclassified bacteria (12.8% and 17%, respectively) [165]. It has been demonstrated that PCR-DGGE short sequences and clone libraries from three stages of *Haemonchus contortus* life cycle (eggs, L_3s, and adults) contained sequences belonging to *Weissella*, *Lactococcus*, *Leuconostoc* and *Streprococcus*[166]. More recent attention has focused on the haemonchine microbiome [167]. The dominant bacterial genera belonged to *Escherichia-Shigella*, *Pseudomonas* and *Ochrobactrum*, which were shared in all the stages of the parasite life-cycle using V3-V4 and V5-V7 amplicons. Moreover, the parasite microbiome could reflect the external micro-organisms (i.e. micro- and macro-habitats).

Conclusion and future directions:-

We have attempted to provide an overview about the high-throughput sequencing and bacteria associated with eukaryotes including parasitic heliminths. There is abundant room for further progress in comparing microbiome of different parasitic helminths, which has, and will continue to offer an important parallel goal for the removal of a wide-variety of devastating animal and human diseases.

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Competing interests:-

The authors declare that they have no competing interests.

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