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A SCITECHNOL JOURNAL

## On the Existence of Animal Viroids

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### Abstract

This manuscript is an outgrowth of my discovery of the viroid in 1971, which has been endorsed by the International Committee for the Taxonomy of Viruses (ICTV) as a new order called *Subviral Agents*, which now consists of two families and upward of 40 species, all isolated from higher plants. Most of these cause diseases of various crops, fruit trees, or ornamental plants.

Here I ask the question why, so far, these plant viroids are not complemented by their counterparts in animals. I explain that mostly two forces are probably responsible for this absence: (1) an excessive anthropocentric bias and (2) a refusal by some to recognize the existence of viroids, as well as of the officially endorsed order of subviral agents. Apparently, no significant efforts were made to discover animal viroids. I propose that well planned research be initiated to study whether animal viroids exist and, if so, whether, in analogy to plant viroids, some animal (human) diseases of unknown etiology may be caused by animal viroids---results which would be of obvious importance.

### Keywords

Viroids; Sub viral agents; RNA; Nucleotides (nt)

### Introduction

The discovery in 1971 [1,2] of the first subviral agent, the potato spindle tuber viroid (PSTVd), triggered the third major expansion in history of the biosphere to include smaller lifelike forms---after the discovery of the "subvisual" microorganisms by Antonie van Leeuwenhoek in 1675 and that of the "submicroscopic" viruses by Dmitri Iosifovich Ivanovsky in 1892. Today, the order of *subviral agents* (so designated by the International Committee for Taxonomy of Viruses, ICTV [3]) consists of two families and upward of 40 species, all of which were isolated from higher plants (except for hepatitis delta virus, which, however, is not a typical viroid, but a much larger, encapsidated RNA [4]).

As cogently expressed by Flores [4], "Viruses (and viroids) share the most characteristic property of living beings: In an appropriate environment, they are able to generate copies of themselves, in other words, they are endowed with autonomous replication (and evolution). It is in this framework where viroids represent the frontier of life (246 to 401 nt), an aspect that should attract the attention of anybody interested in biology" (Figure 1).

Today we know that plant viroids incite a number of damaging

diseases of vegetable crops, fruit trees, and other cultivated and ornamental plants---but where are their counterparts, i.e., subviral agents in extant animals, which, in analogy with plant viruses, may cause animal (human) diseases? Are they non-existent, are they harmless to their hosts (and therefore easily missed), or have they not been searched for diligently enough? Should one accept the apparent conviction of most animal- and medically-oriented investigators that viroids do not exist in animals and that they are therefore of little concern to them?

It is possible, of course, that an unknown number of investigators may have inoculated animals with known plant viroids or, alternatively, tried to isolate subviral pathogens from tissue extracts of apparently healthy animals, or of animals with symptoms of diseases of unknown etiology. If such experiments have been performed, they all must have yielded uniformly negative results, which, by following tradition, would not have been published. Conceivably, therefore, the database to substantiate the conclusion that viroids or other subviral agents are absent in extant animals may exist---but there is no way of knowing.

Here I discuss the very few studies cited in PubMed and/or Google Scholar, in which viroids (or other subviral agents) have been suggested and/or looked for in animals, discuss factors which, based on present knowledge, would increase or decrease the probability of their existence, and finally suggest actions based on these findings.

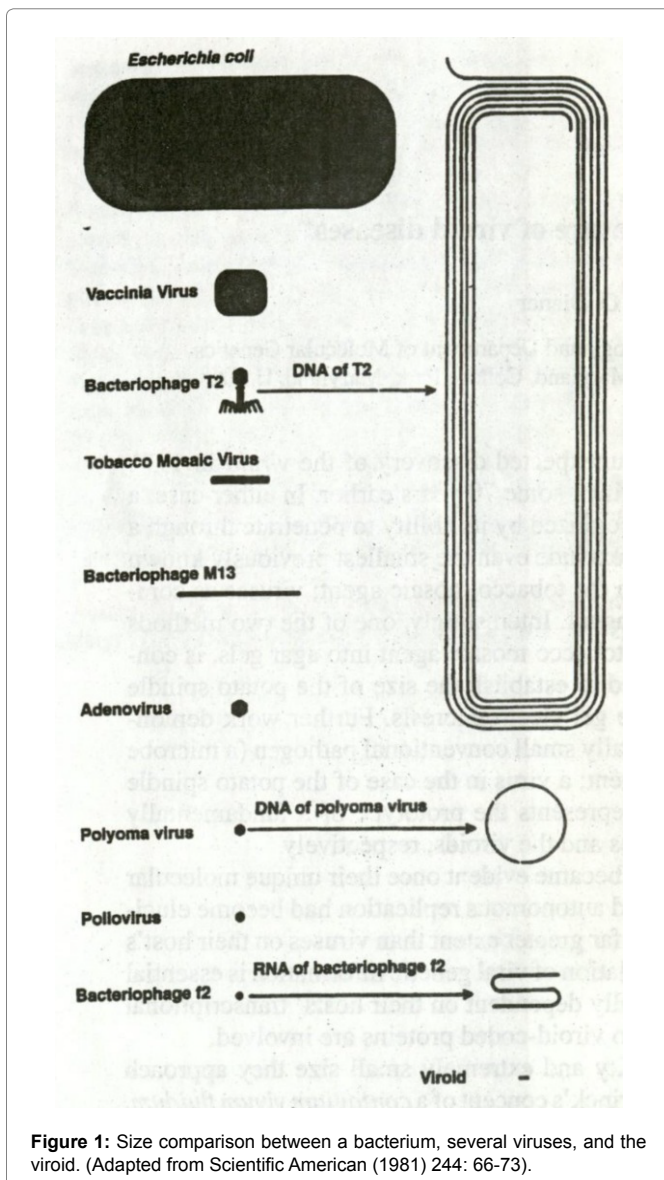
In 1997, Roy [5] stated that "[c]ircular RNAs reminiscent of viroids and the human hepatitis delta virus have been proposed as possible non-conventional pathogens responsible for Crohn's disease and ulcerative colitis---two inflammatory bowel diseases" but, in a carefully performed two-dimensional gel electrophoretic study---in which they used a genuine plant viroid as a control---they failed to detect circular, viroid-like RNAs of conventional size and structure. The authors did identify small (<300 nt), unusually stable, linear RNAs in association with both Crohn's disease and ulcerative colitis tissues. Regrettably, these promising findings seem not to have been followed up, and, today, the underlying causes of these diseases are still enigmatic.

In 2014, Pogue [6] stated that, "as highly soluble and mobile entities, miRNAs possess a highly selected ribonucleotide sequence structure, are part of an evolutionary ancient genetic signaling system, and resemble viroids in their structure, mode of generation and function," as well as that they are very abundant in the physiological fluids that surround cells and tissues. The authors stated "that persistence and altered abundance of miRNAs in the extracellular fluid (ECF) or cerebrospinal fluid (CSF) may play a role in the intercellular spreading of disease systemically, and throughout functionally-linked cellular and tissue systems, such as the central nervous system (CNS)." The authors speculated on the presence of these highly structured, single-stranded RNAs in the human CNS, with particular reference to Alzheimer's disease.

In conclusion, there seem to have been exceedingly few efforts to find viroids in animals---whether or not associated with diseases. Although, at first glance, the extremely low level of experimentation in efforts to discover animal viroids may seem surprising, it is

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Received: August 26, 2016 Accepted: September 19, 2016 Published: September 27, 2016



**Figure 1:** Size comparison between a bacterium, several viruses, and the viroid. (Adapted from Scientific American (1981) 244: 66-73).

understandable considering that the very concept of subviral agents is not familiar to most animal and medical investigators. Also, as stated by Zhang [7], “The rate of discovery of these subviral plant pathogens was low over the past 40 years because the classical approaches are technical[ly] demanding and time-consuming”---circumstances, which may have discouraged experimentation in efforts to discover animal viroids.

There still exist other forces, however, which may have drastically limited the extent of experimentation with subviral agents by animal- and, above all, by medically- and biochemically-oriented investigators.

For example, many investigators adhere to an excessive anthropocentric bias, which automatically downgrades the significance of results obtained with plants, in disregard of the fact that many important biological discoveries have first been achieved with plants or plant tissues. Suffice it to mention here Gregor Mendel’s fundamental laws of genetics and the discovery of the first virus,

tobacco mosaic virus, as well as determination of its molecular constitution---all of which were first achieved with plants or plant systems. It was thus useful for Ding [8] to remind investigators that viroids are, aside from their pathogenic properties, most useful systems to study basic principles of RNA biology.

This bias is well documented by the virtual absence of the terms *viroid* and *subviral agent* in the animal and medical literature, at times when plant virologists and plant molecular biologists had already made great strides in elucidating the unique molecular properties and modes of replication of viroids, producing important results with significance not only to virology, but to science in general, such as the first complete nucleotide sequence of an eukaryotic RNA pathogen, potato spindle tuber viroid [9].

Even an extreme anthropocentric bias alone, however, seems insufficient to explain denial by some authors of the very existence of subviral agents, as exemplified by Cech [10], who, as recently as in a 2015 retrospective of “RNA World research” mentions long, non-coding RNAs, but not subviral agents or viroids---the discovery of which has been declared by the American Phytopathological Society to be one of the ten most important, pathogen-related discoveries in plant pathology of the 20<sup>th</sup> century [11].

Without doubt, the two factors of anthropocentric bias and denial of the existence of plant subviral agents by biochemically- and medically- oriented investigators combined to drastically reduce efforts to discover animal subviral agents in extant animals.

## Conclusion

While my survey did not disclose evidence of an existing animal subviral agent, neither do the few negative reports listed permit drawing the conclusion that such agents are absent in animals. Only much further experimentation could provide definite evidence one way or other. Such evidence would be much easier to obtain today than was the case during the last 40 years, for example by use of novel methods which can analyze multiple sample simultaneously [7]. Whether such additional efforts should be undertaken is not so much a scientific decision, as one of expected returns, i.e., whether it is deemed important to know whether animal subviral agents exist and, if so, whether some may incite animal (human) diseases. Surely, such considerations would greatly enhance the importance of acquiring as much knowledge of subviral agents as possible.

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