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**CONTENTS**

Editorial Board  
Notes for Contributors

Inside Front Cover  
Inside Back Cover

**Articles**

**Social Sciences**

- A Review of Entrepreneurship and Rural Growth: Current Issues and Practices 3  
*George M. Korres, Efstratios Papanis and Aikaterini Kokkinou*
- Between Theory and Practice: Investigating the Institution of Surveillance 21  
in the Framework of Preschool Education  
*Sidiropoulou Trifeni, Sidiropoulou Maria and Anna Barkia*
- US Employment Laws in a Multi-National Environment: 35  
A Comprehensive Analysis  
*Andreas Petasis*

**Applied Sciences**

- Surface Interactions and Cellular Uptake of Metal Nanoparticles in 51  
Primary Lymphocyte Cultures - Brief Report  
*Katerina Todorova, Ani Georgieva, Anna Dikovska,  
Reneta Toshkova, Rositsa Milcheva, Boicho Nikolov,  
Vladimir Kozlov, Modra Murovska and Russey Russev*
- Evidence of Productive HHV-6 Infection in Autoimmune Thyroiditis Patients' 59  
Thyroid Gland Tissue Samples  
*Katerina S. Todorova, Alina Sultanova,  
Maksims Cistjakovs, Egils Cunsksis,  
Rositsa S. Milcheva and Modra Murovska*



## A REVIEW OF ENTREPRENEURSHIP AND RURAL GROWTH: CURRENT ISSUES AND PRACTICES

GEORGE M. KORRES<sup>1</sup>, EFSTRATIOS PAPANIS<sup>2</sup> and  
AIKATERINI KOKKINO<sup>3</sup>

### ABSTRACT

*The importance of entrepreneurial activities for economic growth has been emphasized by economic literature. Entrepreneurship can be considered as the driver of economic growth and local development (the so-called “engine of growth”). The impact of entrepreneurship is affecting the employment and competitiveness level as well as growth process. Much of the recent work on economic growth can be viewed as refining the basic economic insights of classical economists. The recent debate on the determinants of output growth has concentrated mainly on the role of knowledge typically produced by a specific sector of the economy, and furthermore on the role of entrepreneurship and the implications on economic growth. In the past, public policy tended to focus on rural areas as a block, treating them homogeneously with uniform problems and opportunities. However, the unit of analysis and intervention has dramatically and such an approach no longer reflects the present changed development of rural areas. Rural policy has had significant developments the last two decades. This paper aims to review and analyze the entrepreneurship and rural policy and its effects on rural and economic growth.*

**Keywords:** Entrepreneurship; Rural; Economic Growth; Competitiveness.

### 1. ENTREPRENEURSHIP AND ECONOMIC GROWTH

An entrepreneur is an individual who assumes the financial risk of developing or managing a new venture, where the venture is based on a new idea or an innovative way of performing a task. The “entrepreneurial spirit” is something that has long been associated with the driving force behind economic progress and growth. Joseph Schumpeter (1950) stated that the key to the success of markets lies in the spirits of entrepreneurs who persist in developing new products and technologies, and succeed at ultimately reducing production costs. Entrepreneurship is the driver for economic growth in Europe’s rural areas. Entrepreneurship is one of the main key-components of the EU’s Europe 2020 strategy for smart, sustainable and inclusive growth. Furthermore, the so-called “entrepreneurial networks” may be defined as these groups

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of interconnected entrepreneurs that aim to disseminate information and ideas, in order to enhance their businesses. These networks can also access financing activities, find business partners, and get new ideas for new products. Entrepreneurs gain multiple benefits from their networking activities, including an expansion of their resource base, learning about business processes and opportunities, and generating reputation and profits.

The last two decades have witnessed a wealth of studies analyzing the *determinants* of entrepreneurship. While some of these studies are theoretical (Holmes and Schmitz, 1990), others are empirical (Evans and Leighton, 1990). The *consequences* of entrepreneurship, in terms of economic performance, have also generated an extensive literature. However, this literature has generally been restricted to two units of observations – that of the establishment or firm, and that of the region. Noticeably absent are studies linking the impact of entrepreneurship on performance for the unit of observation of the country. A large literature has emerged analyzing the impact of entrepreneurship on economic performance at the level of the firm or establishment.

The non-agricultural sector is economic activity in urban or semi-urban areas. People who live in these areas are involved in industrial enterprises, both at the production and managerial level as well as in various service sectors, such as trade and tourism. Although the norms and regulations vary from country to country, the firms of urban sector (or otherwise the *formal-sector*) receive the benefits of state economic support. Usually, the firms on this sector operate under the accepted rules and regulations imposed by the government. The workers of these firms belong to a union and collective bargaining between firms and workers is not uncommon. The firms are required to pay minimum wages and to conform to certain standards such as, safety, pension schemes and so on. These firms pay taxes and may receive infrastructural facilities, such as the access to subsidized electricity, and may have access to foreign exchange quotas or the right to import certain inputs. In contrast, the agricultural sector (or otherwise the *informal sector*) usually has small-scale organizations and lower basic infrastructure. This sector usually does not adhere to norms of minimum wages, retirement plans or unemployment compensation. Usually, firms do not pay taxes and they receive little government support. People in agriculture are often very poor and they face high levels of risk, while the primary occupation in agricultural sector is farming.

Explanations for economic growth have generally been restricted to the realm of macroeconomics (Romer, 1990; Krugman, 1991). However, a different scholarly tradition linking growth to industrial organization dates back at least to Schumpeter (1934). According to this tradition, performance, measured in terms of economic growth, is shaped by the degree to which the industry *structure* utilizes scarce resources most efficiently.



Brock and Evans (1986) argue that the shift away from large firms is not confined to manufacturing industries, and provide four more reasons why this shift has occurred:

- the increase of labour supply leading to lower real wages and coinciding with an increasing level of education;
- changes in consumer tastes;
- relaxation of (entry) regulations; and
- the fact that we are in a period of creative destruction.

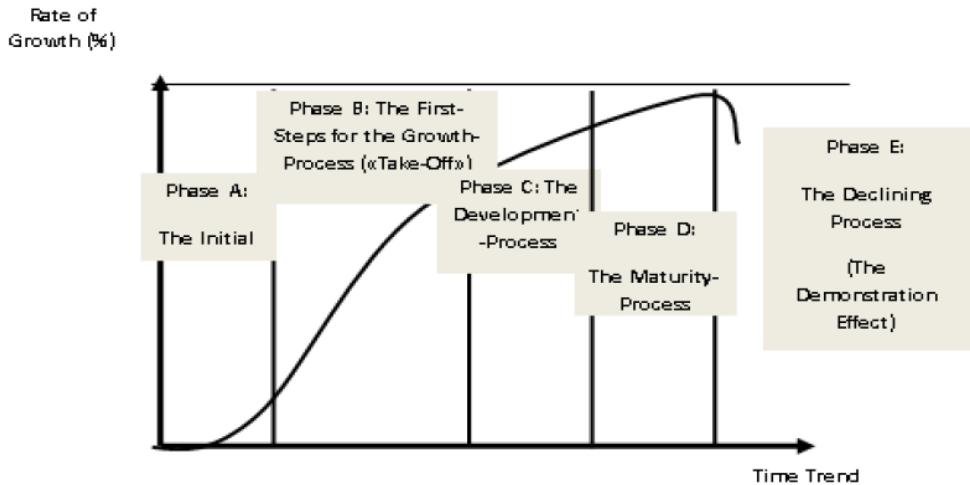
Loveman and Sengenberger (1991) stress the influence of two trends of industrial restructuring: that of decentralization and vertical disintegration and that of the formation of new business communities. These intermediate forms of market coordination flourish owing to declining costs of transaction. Furthermore, they emphasize the role of public and private policies promoting the small business sector.

ACS (1992) was among the first to discuss them. ACS distinguishes four consequences of the increased importance of small firms:

- entrepreneurship
- routes of innovation
- industry dynamics and
- job generation

ACS claims are that small firms play an important role in the economy serving as agents of change by their entrepreneurial activity, being the source of considerable innovative activity, stimulating industry evolution and creating an important share of the newly generated jobs. The role of small firms in the job creation process remains controversial. The re-evaluation of the role of small firms is related to a renewed attention to the role of entrepreneurship in firms. Figure 1 indicates the role of entrepreneurship and the implications in the growth process, according to the Schumpeter's lines in theory of the role of entrepreneurship on the growth-process.

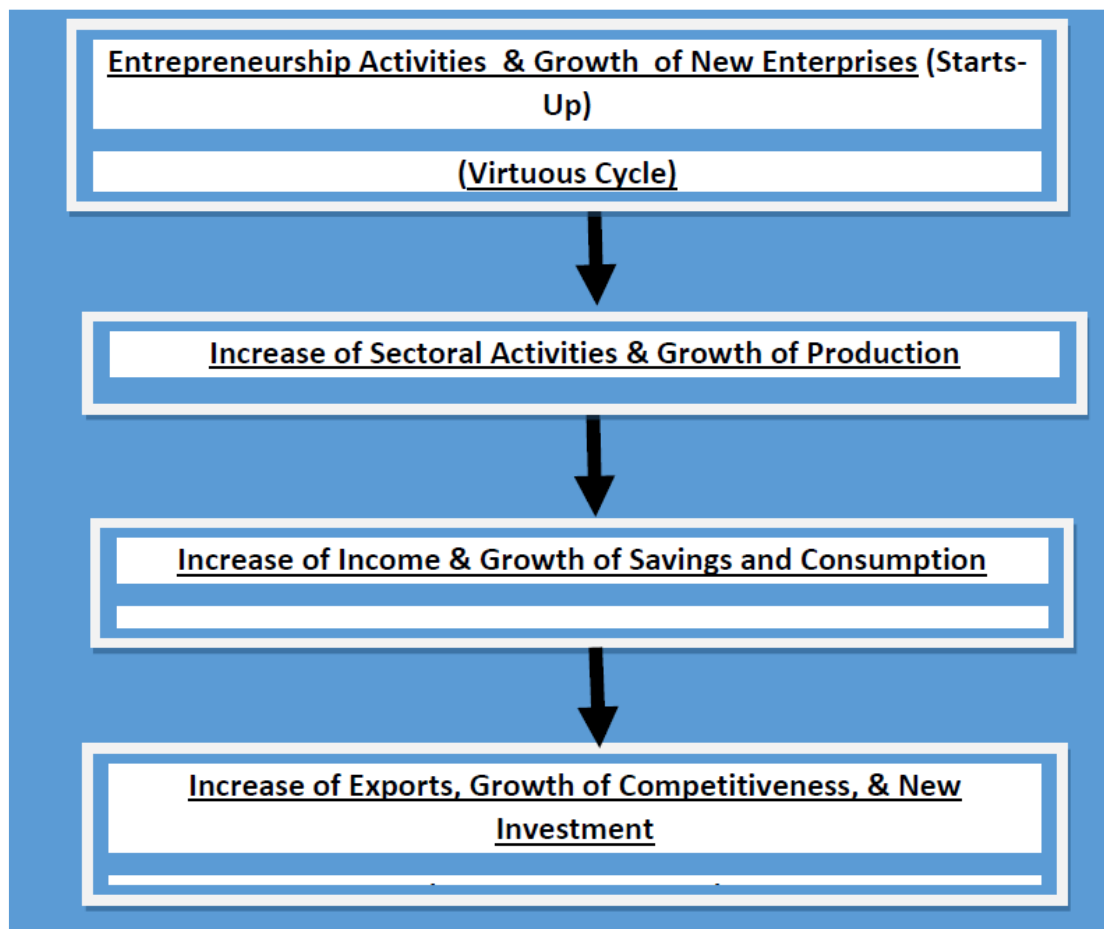
**FIGURE 1: ENTREPRENEURSHIP AND THE GROWTH PROCESS**



*Source: Authors' Elaboration*

Entrepreneurship has to do with activities of individual persons. The concept of economic growth is relevant at levels of firms, regions, industries and nations. Entrepreneurship is not synonymous with small business. Certainly, small firms are an outstanding vehicle for individuals to channel their entrepreneurial ambitions. The small firm is an extension of the individual in charge (Lumpkin and Dess, 1996, p. 138). Schumpeterian entrepreneurs are found mostly in small firms. They own and direct independent firms that are innovative and creatively destroy existing market structures. Schumpeter emphasizes the role of the entrepreneur as the prime cause of economic development. He describes how the innovating entrepreneur challenges incumbent firms by introducing new inventions that make current technologies and products obsolete. Managerial business owners, entrepreneurs in a formal sense, are to be found in the large majority of small firms. Recently, the conceptual link between entrepreneurship and economic growth has received renewed interest by economists. The finding that increased entrepreneurial activity leads to greater economic growth is now well founded at both national and local level. The purpose of this article is to provide a survey of what is known about the links between entrepreneurial activity and rural economic growth. Figure 2 illustrates the role and the implications of entrepreneurial activities in competitiveness and growth level (the so-called virtuous cycle phenomenon).

**FIGURE 2: THE IMPLICATIONS AND THE EFFECTS OF ENTREPRENEURIAL ACTIVITIES**



*Source: Authors' Elaboration*

## 2. RURAL POLICY AND THE GROWTH PROCESS

An examination of rural policy requires an understanding of the unique conditions in rural places that justify separate national policies. There are several characteristics of rural areas that make them relatively unique and therefore justify special policies. The most of these is relatively low population densities, which create fewer organizational resources for most activities. Population density also makes it difficult to achieve economies of scale in the provision of services and therefore cause many costs to be

higher and require people and organizations to be less specialized. Of course, the exception is the heavy specialization of many rural areas on single natural resource oriented industries, like agriculture, mining and energy. These industries have often had profound effects on rural areas because they have concentrated resources in a few hands and contributed to the concentration of economic and political power and therefore have weakened democratic and civic institutions. Rural development policy also contributes towards the objective of social and territorial cohesion within the European Union.

The rural policy should focus on enforcing the entrepreneurial activities, under the four main headings:

- (1) Tools to support the rural entrepreneurship
- (2) Emerging sectors for the rural economy;
- (3) Overcoming obstacles to entrepreneurship;
- (4) Social aspects of entrepreneurship

A major subject of rural policy related to the basic infrastructure is the remote areas. Usually, rural policy in remote areas should provide those services necessary to meet the demand of users and also to enhance the whole basic infrastructure and to improve the quality of life. Due to isolation of the remote areas, rural policy on these areas should affect them directly and remote areas will gain more from the upgrading of basic infrastructure, like for example from roads and telecommunications. However, the indirect effect of rural policy on particular remote areas, such as on employment, education and training, are not so strong due to luckiness of skilled labor and the low level of investment and of the basic infrastructure.

Furthermore, rural entrepreneurs face problems associated with:

- the long-term decline in agricultural incomes
- supportive actions for entrepreneurship-networking
- transportation problems and greater distances to global markets;
- the small size of local enterprises
- the limited local demand and the low population densities of rural communities

The existing patterns of supporting economic activity in rural areas focus predominantly on agriculture and food processing. Agriculture and rural development are expected to contribute to the Europe 2020 strategy. The role in supporting entrepreneurship in rural areas lies in the hands of local governments in order to enhance the growth process and the results expected from supporting entrepreneurship are:

- an increase in overall employment;
- increase in employment outside of agriculture;
- increase in demand;
- increase in the value of investments;

- a larger tax base and, as a result, an increase in the revenue collected by local authorities;
- diversification of economic activity and thus a lower dependence on market volatility;
- strengthening and an expansion of existing enterprises;
- an increase in the number of investors; and
- an increase in the number of enterprises.

### 3. POLICIES FOR ENTREPRENEURSHIP AND RURAL GROWTH

There are various dimensions to thinking about entrepreneurship and enterprise policies that we need to take into account when defining the scope of the policies that this paper is concerned with. In this regard, we may distinguish between on the one hand policies to encourage and support entrepreneurship, such as entrepreneurship policies which include the promotion of an entrepreneurial culture, entrepreneurship education, and policies to help individuals through the nascent and initial stages of starting a business (Stevenson & Lundstrom, 2002), and on the other hand, more traditional enterprise support policies concerned with the growth, survival and competitiveness of existing SMEs. There are many different factors which can influence the level of entrepreneurship within a rural economy, not least the specificities of the social, cultural and political context including national level policies with regards to, for example, market openness, privatization, and legal and taxation regimes (Glancey & McQuaid, 2001; Morrison, 2000; Reynolds et al., 2002). However, we focus here upon those entrepreneurship policies which are directly concerned with building-up the entrepreneurial capacity of rural regions. These include:

- (i) Policies concerned with building the entrepreneurial capacity of rural regions such as those seeking to influence the attitudes and motivations of individuals towards entrepreneurship and providing opportunities for the acquisition of business and management skills via the education and training process;
- (ii) Policies concerned with targeting potential sources of entrepreneurs, such as those aimed at attracting immigrants with entrepreneurial skills and ambitions or those initiatives aimed at increasing the proportion of entrepreneurs from “under-represented” groups such as young people or women;
- (iii) Policies concerned with supporting the process of starting new business ventures, including pre-start-up advice and appraisal of the “business idea” as well as assistance with the various aspects of setting up a new business.

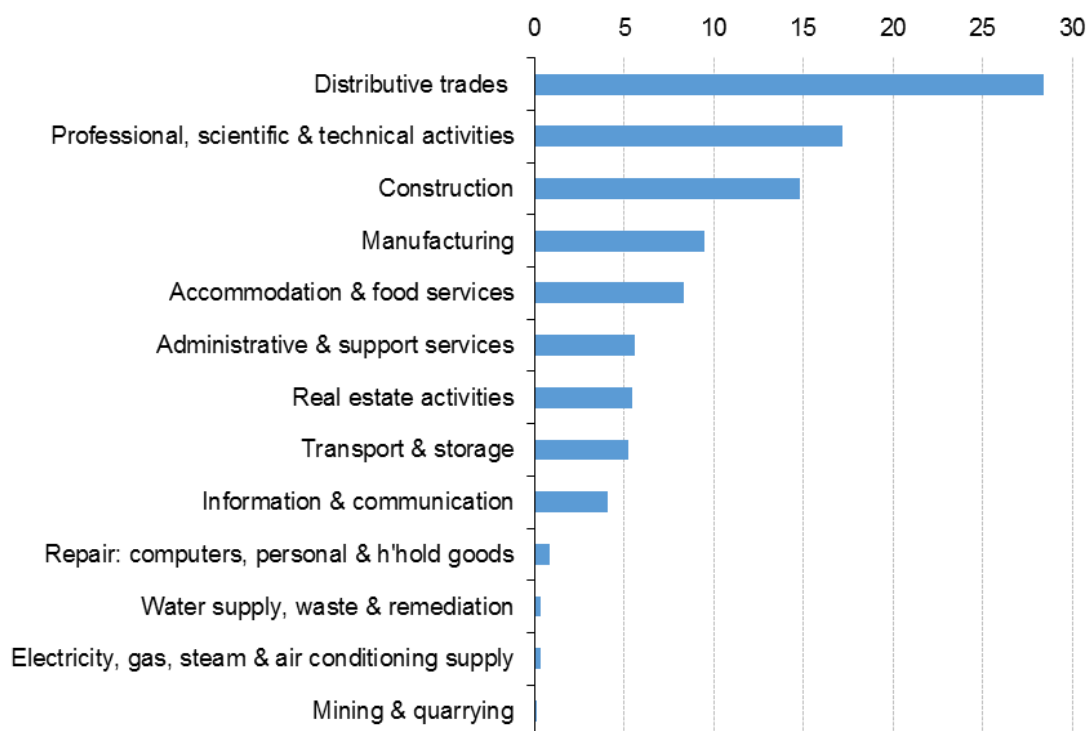
More generally, enterprise support policies are primarily concerned with improving the competitiveness and viability of existing SMEs with a view to increase their chances of survival and growth. These include:

- (i) Policies concerned with generic support to rural businesses, such as those concerned with providing advice on different aspects of running a business, such as business planning, marketing, exporting, use of information and communication technology.
- (ii) Policies aimed at providing specialist support to enterprises in particular sectors, such as those initiatives concerned with helping farmers;
- (iii) Policies concerned with providing an infrastructure which is supportive to enterprise formation and development in rural regions.

#### 4. EVIDENCE FROM RURAL POLICY AND ENTREPRENEURSHIP ACTIVITIES

The great differences among rural people and places in most countries make it hard to discover national policies that fit all of these places. Isolated rural areas will generally exist at considerable distance from urban centers. These communities will be those that survived a period of significant rural consolidation. Population will be stable or declining. Income levels will be significantly lower and income growth will lag behind the national average. The basic infrastructure on these communities, including transportation and telecommunication infrastructure, will be typically less (at least one generation) behind that of urban and growing rural areas.

**FIGURE 3: BREAKDOWN OF NUMBER OF ENTERPRISES WITHIN THE NON-FINANCIAL BUSINESS ECONOMY, EU-28, 2014**



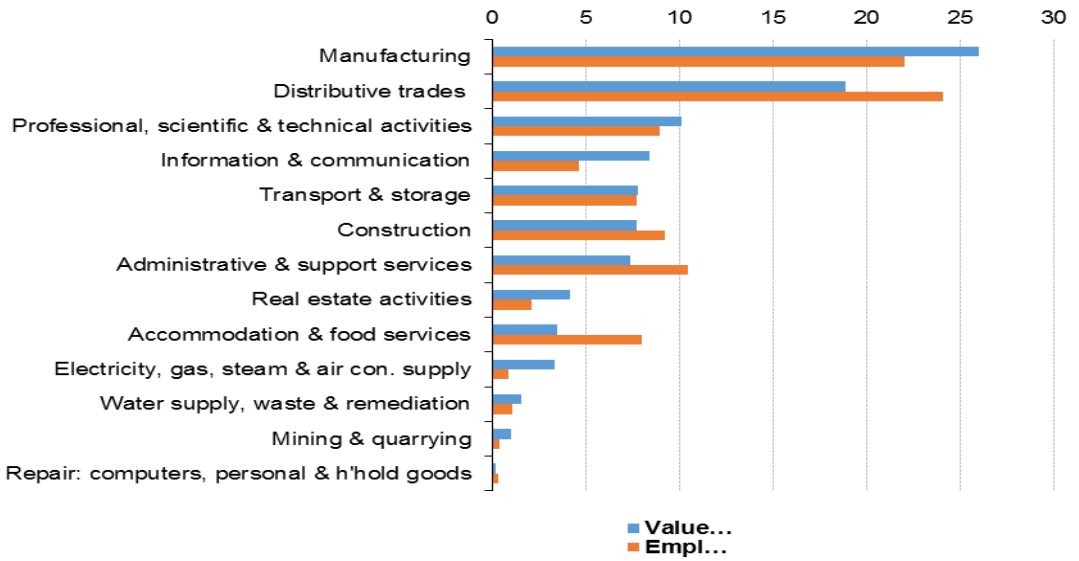
*Source: Eurostat Database*

The policy orientation is closely related to the main socio-economic factors, the basic priorities and the emerging needs. The main macroeconomic and social factors in relation to the rural policy are presented on the following Figures 1-5. In particular, Figures 3 and 4 illustrate the breakdown of number of enterprises within the non-financial business economy, EU-28, and also the analysis of non-financial business economy value added and employment, EU-28, respectively. Figure 5 illustrates the European productivity and Growth GDP, per capita income and labour productivity, respectively. However, “macroeconomic policies”, dealing for instance with national growth, stable prices and financial system, as well “structural policies”, concerning for example the efficiency of markets, will not be sufficient to deal with new and more intense rural problems.

In rural areas, economies of scale are more likely to be achieved internally to firms and consequently firms are becoming increasingly larger and larger. Evidence from the agricultural sector is the emergence of supply chains, whereas in urban areas

small to medium firms can cluster to capture the benefits of agglomeration economies, as the saving due to proximity to a diverse labor force, specialized producer services and high-quality public services.

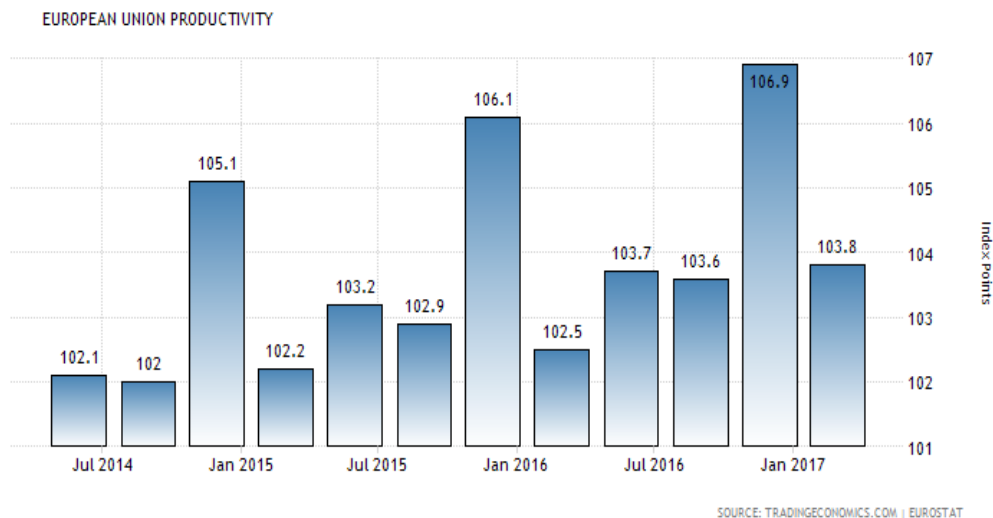
**FIGURE 4:** ANALYSIS OF NON-FINANCIAL BUSINESS ECONOMY VALUE ADDED AND EMPLOYMENT, EU-28, 2014



*Source: Eurostat Database*



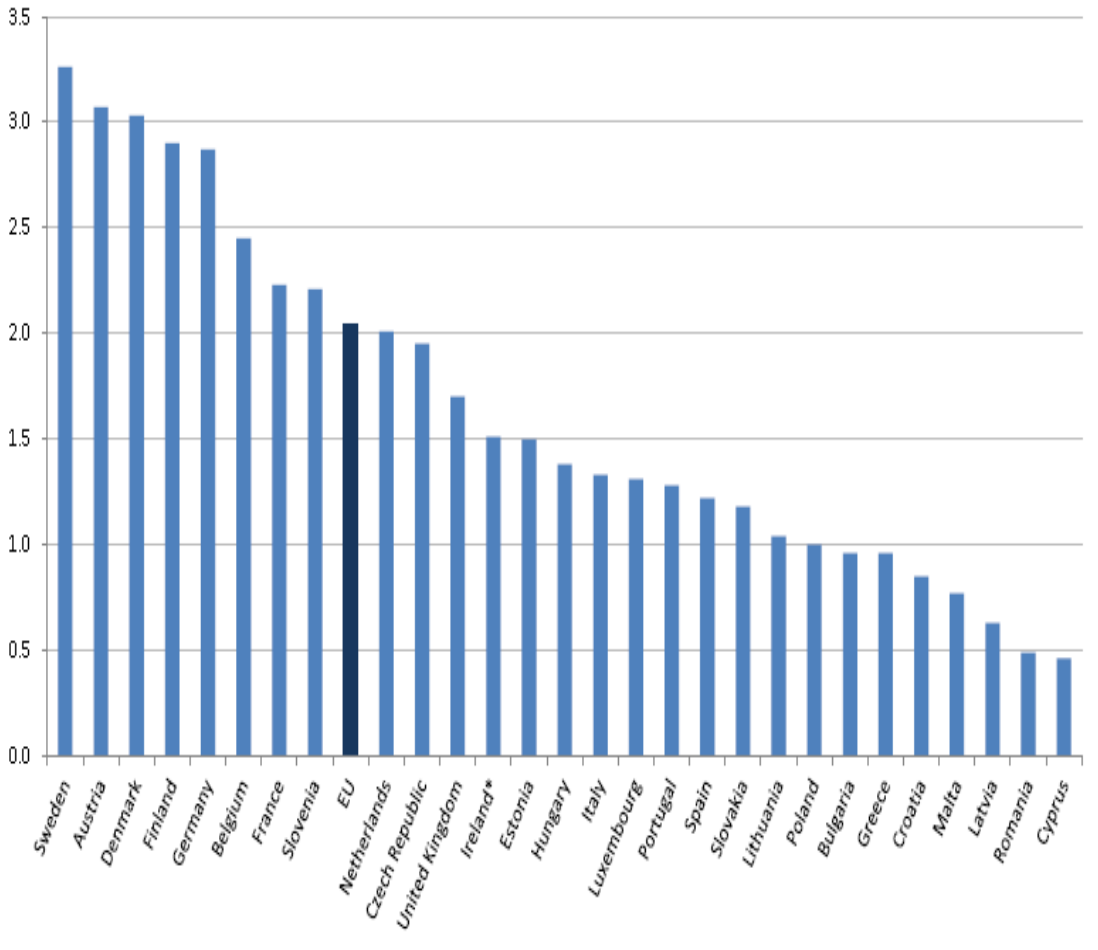
**FIGURE 5: THE EUROPEAN PRODUCTIVITY**



*Source: Eurostat Database*

Unequal distributions of wealth and income have created rural poverty, which is likely to be different from urban poverty. In urban areas more poor people are single-parent heads or households. In rural areas, they are more likely to be the working poor. Therefore, employment and training programs are likely to do more for rural than urban poverty. Improved rural development has often been associated with greater capital investment, the application of science to production, better economic organization, and in some cases effective urbanization. The notion is that infrastructure is public capital investment that will make private capital investment more productive.

**FIGURE 6: R&D INTENSITY IN THE EU MEMBER STATES, 2015:  
(R&D EXPENDITURE AS % OF GDP)**



*Source: Eurostat Database*

The expectation is that improved water, better electricity, lower cost transportation, and augmented information infrastructure in rural areas can allow firms to be more productive and operate at lower costs. Figure 6 illustrates R&D intensity in the EU Member States, 2015 (R&D expenditure as % of GDP) and Figure 7 illustrates the R&D intensity in the EU Member States, 2015 (R&D expenditure as % of GDP), respectively.

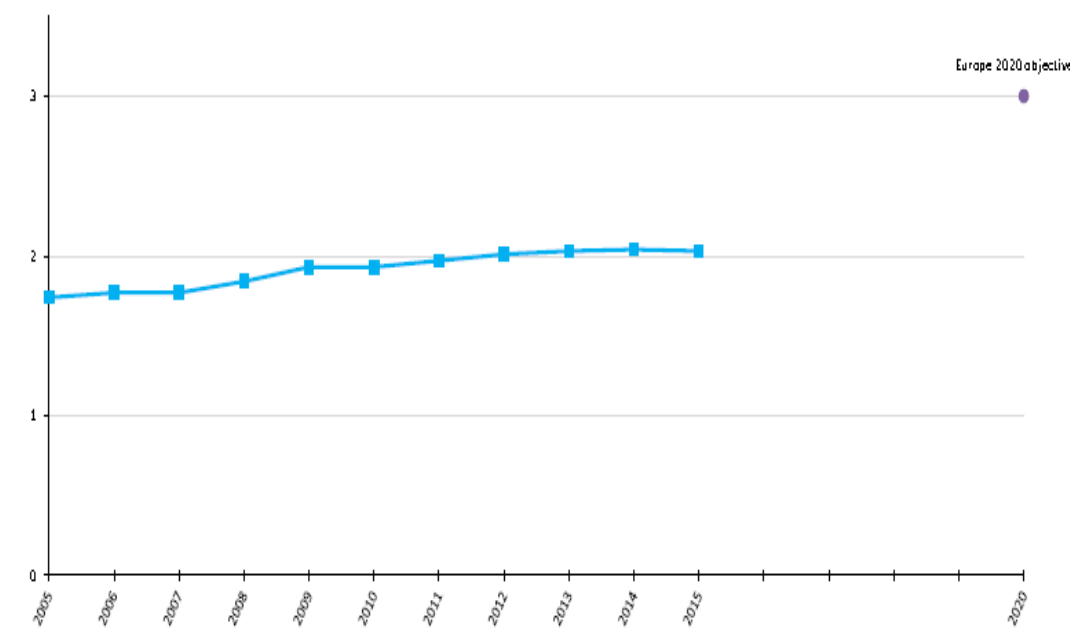
Entrepreneurship and innovation are the key components of the EU's Europe 2020 strategy for smart, sustainable and inclusive growth. Business innovation is quite

important and can also affect the agricultural sector and the related activities, such as the food-production and marketing. Figure 8 illustrates the main pillars, the “system of four-axes”, for the growth-process.

The resulting productivity gains are expected to increase overall economic activity. A range of tools can potentially be used to make rural areas more economically vital.

The challenge for policymakers, functioning with limited resources at their disposal, is to select the mechanisms that are most efficient for stimulating rural economies. In this way, infrastructure is best seen as one of the competing means for enhancing rural economic development. For instance, water, electricity, telecommunications and other infrastructure are obviously imperative to business development.

**FIGURE 7: RESEARCH AND DEVELOPMENT INTENSITY IN THE EU,  
2005-2015**  
(R&D EXPENDITURE AS % OF GDP)



Source: Eurostat Database

**FIGURE 8: POLICY TOOLS AND THE MAIN AXES FOR THE GROWTH PROCESS**



*Source: Elaborated by the authors*

During the last two decades, rural policy focuses on the following points:

- There was an attempt to pursue strategic investments and to develop new activities for basic infrastructure.
- There was more attention to public investment and to support local enterprises, local entrepreneurship, and innovation, as well as to assure social cohesion.
- There was a clearer planning for exploitation of local resources and to develop the comparative advantage.
- There was a support on sectoral policies aiming to enhance the inter- and –intra linkages effects at sectoral, local and regional levels.

- Finally, there was an effort to increase the decentralization of administration and policy and furthermore to increase the partnership between public and private sectors, in order to improve and to increase the necessary basic infrastructure of the rural regions.

## 5. CONCLUSIONS

Promoting and strengthening entrepreneurship is one of the most important pillars of enforcing the process of economic growth. Moreover, rural entrepreneurship continues to attract attention and to help rural areas and the local development. Enterprise and entrepreneurship are the drivers of economic growth in Europe's rural areas. We expect a framework relating entrepreneurial activity to economic growth to hinge on at least four elements.

- *First*, on the literature identifying the micro-economic foundations of growth emphasizing the role of knowledge externalities in the growth process (Romer, 1986 and 1994).
- *Second*, it should identify intermediate linkages.
- *Third*, it should deal with dual causality in the relation between entrepreneurial activity and growth.

The main factors that are favourable to entrepreneurship and investment in rural areas are:

- *Loyalty of employees*: Because it is difficult to find a job, and work alternatives are scarce, having a job is highly appreciated.
- *Lower crime rates*: The safety of running a business is an important factor that investors take into consideration before making an investment decision.
- *Ease of adopting new consumer behavioural patterns*. Entrepreneurial and market-oriented attitudes are slowly adopted by people in rural areas but this is not the case with consumer behavioural patterns.
- *Clean environment*: Clean air, low noise levels, and unpolluted water are advantages.
- *Easier control of employees*: Lower levels of education and a smaller number of work alternatives make it easier for employers to control workers.
- *Lower prices of land*: Lower land prices can attract investors willing to build an enterprise.
- *Lower wages*: The cost of living is substantially lower in rural areas than elsewhere and wages can also be relatively lower.
- *Negligent role of trade unions*: Although workers have a right to form trade unions, they seldom make use of that right in rural areas.

The main factors that are unfavourable to entrepreneurship and investment in rural areas are:

- *Low population density:* Because of low population density, human settlements are far from one another, and communication is slow.
- *Low demand per square kilometre:* Market potential, which denotes the volume of goods sold in an area, is one of the basic measures used in marketing.
- *Large distances:* As a result of low population density, human settlements are scattered over a large area. Large distances increase the cost of transportation and reduce the frequency of supply deliveries.
- *Lack of an entrepreneurial tradition:* Entrepreneurship, coming up with new ideas and innovations, and actively trying to overcome obstacles and difficulties.
- *Low level of education:* The level of education can heavily influence a company's decision about where to invest.
- *Lack of models for successful business ventures:* Instances of successful business undertakings are an enormous encouragement for other people to do the same. In rural areas, however, such examples of successful entrepreneurship in rural areas undertakings are rare.
- *Problems with infrastructure:* Underdeveloped infrastructure can scare off potential investors.

Entrepreneurship generates growth because it serves as a vehicle for innovation and change. One of the central goals of public policy common among all modern economies is the generation of growth and the creation of employment. Much of the policy debate to generate growth and jobs has relied on a macro-economic framework and focused on the traditional macro-economic policy instruments. The survey of this article suggests that a different, less traditional instrument for generating growth and employment plays an important role – policies that generate and promote entrepreneurship (OECD, 1998). Empirical evidence surveyed in this chapter suggests that those countries that have experienced an increase in entrepreneurial activity have also enjoyed higher rates of growth. Experience also suggests that an approach which actively involves rural communities, enterprises, and economic development agencies is most likely to work best, although regional level economic development organisations are probably in the best position to achieve the level of integration between different interests and agencies which will be required.

Based on the increasing awareness of the role of entrepreneurs in driving economic growth, state and local economic development efforts have been more heavily directed toward promoting entrepreneurship. These development efforts have mainly focused on reducing the financial constraints that entrepreneurs face either through preferential loans to new businesses, as those supported by the Small Business Administration, or preferential tax treatment for new or small businesses. One such policy that has recently gained popularity is to devote public resources toward attracting and building a larger amount of venture capital to encourage entrepreneurial activity. This development strategy is largely based on casual observation that areas

with larger amounts of entrepreneurial activity generally tend to also have a larger amount of venture capital.

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## BETWEEN THEORY AND PRACTICE: INVESTIGATING THE INSTITUTION OF SURVEILLANCE IN THE FRAMEWORK OF PRESCHOOL EDUCATION

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### ABSTRACT

*Various perspectives based on the experiences of future childhood pedagogues on subjects that are presented of their training, focusing mainly on how to be able to withstand supervision during the practice of laboratory exercises. A student interview was used to collect the data of (8th) semester of the Preschool Education Department of TEI of Athens. Data analysis and sources have highlighted the need for supervision, the characteristics that the supervisor must possess to be labeled "effective," the reasons why the students needed his presence and the feelings that they experienced with him in their first pedagogical applications at the site of its implementation practice.*

**Keywords:** *Preschool Pedagogues; Supervision; Students; Internship.*

### 1. INTRODUCTION

The text focuses on investigating the need for a trained person to support students in their first pedagogical experiences to acquire their professional identity. The research participants were students from the Department of Preschool Education of the Athens Technological Educational Institute who had the experience of supervision during their compulsory internship at kindergarten in the context of their studies (Pre-School Study Guide Conduct, 2017). The individual research questions are aimed to identify the characteristics of the supervisor-mentor that contribute to make one effective supervision. At the same time, they are exploring forms of communication students choose to have with the supervisors-mentors while also looking for the reasons to search for a supervisor-mentor to support students. Finally, they focus on the thoughts and feelings of the students in the presence of the supervisor-mentor in the implementation of their pedagogical efforts.

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The purpose is to record and explore the experiences of his students who belong to the Department of Preschool Education in Athens regarding their support from people in the framework of their training at the time they were doing practical application of their knowledge and acquired a professional identity. Moreover, material was collected through discussions. As the research moves around role of support and guidance it is important to note that the use of the term "supervisor" is conceptually identical to the terms "mentor", "instructor", "responsible trainee" "Assessor-Examiner". All the above scenarios are related to the person they support, proposes solutions, collaborates, communicates with students throughout their lives first pedagogical experiments. This role is more complex, since in addition to the above responsibilities, it strengthens, guides, promotes, evolves and mainly promotes the theory-practice link through experience and training of (Salvaras, 2013).

## 2. LITERATURE REVIEW

The existing literature consists of a mix of studies with mentoring aimed more about supporting the professional development of the newly appointed professional (Hobson, Ashby et al, 2009) and not in the context of drawing up future pedagogues. Recently, however, a collective work of members of the Practice Exercise Network of Preschool Education Departments has been presented (Avgetidou, Tzakaki and Tsafos, 2016) where theoretical frameworks, processes, methods and tools support prospective pedagogues in their internship. Emphasis is placed on supporting the creation of retreating professionals, collaborating teachers who research and constantly seek to improve their professional identity. What is more, the concept of mentorship as Stravakou claimed (2007) is exclusively linked to the safe and integrated development and development of the potential of the person under his / her own protection. He also points out that today the importance of the pre-mentioned concept emphasizes the professional and personal development of the apprentice. It corresponds to the one who has a positive influence, aims at removing the apprentice from isolation environments and helping him with special guidance to accomplish high goals.

Mavrogiorgos (1996) emphasizes that the transition period of the educator from basic education to the exercise of his duties is the most difficult and important period for his personal and professional development. He points out that drastic changes in his orientations, ideas and behavior have taken place during this period. Matsangouras (2005) believes that by providing assistance and support, educators will be able to cope with school problems and develop personal ways of approaching them. While in describing the Greek school reality, which is something complex and constantly changing, the most important factor of its quality is the educator with his knowledge, his perceptions and his professional development (Mlekanis, 2005). The Greek bibliography seems to face a mentor as the person who is called upon to help

the trainees connect the new knowledge with their existing personal experiences and perceptions, offering them a new perspective (Deligianni, Matthewaki, 2008).

The importance of a mentor is additionally evident in the international literature, where it has been frequently reported and realized in recent years (Stravakou, 2007) and has expanded its scope. Lim (2005) is describing the role of the mentor as someone who denotes and helps the positive development of another person. He acts with the attributes of the parental form, the teacher, the standard behavior, the accessible and trusted advisor on not only professional matters, but also issues of life. He is synonymous with the promoter, the ally, the assistant, the professional instructor of the person who has undertaken to be next to him. The literature seems to support that the mentor-trainee relationship can have positive effects on the professional development of the teacher (Dalozi, 1986). This takes place since the mentor aims at the holistic development and continuous improvement of the trainee in a climate of open, honest communication and mutual respect. Furthermore, the appropriate challenges that are compatible and relevant to the trainee's needs are provided. It is clearly stated that within the mentor institution a specific chemistry emerges between the two individuals, mentor-guided, aiming at the achievement of the goals of professional development and their personal development (Knippelmeyer & Torracio, 2007). Taking this further, a mentor is a constant source of help and support, a good listener, allowing reflection and giving opportunities, offering emotional support and knowledge of communication skills. At the same time he should move in a reliable and accessible way, helping to create a comfortable environment to develop (Rippon, Martin, 2006).

Finally, the difficult transition from initial teacher education to entry into the new workplace should be smoothed out by the mentor, Sundli (2007) points out that a mentor, guides, reassures and is committed to boosting self-confidence and self-esteem. As the author argues, this institution aims both at the continuous personal and professional development of the teacher.

### 3. METHODOLOGICAL APPROACH

The investigation took place in the period of April October 2016 and lasted 6 months. The sample used in research (symptomatic and selected), were 11 university students of Department Technological Educational Institution (TEI) of Athens 8th semester. The methodological tools which were used are open release interview (Cohen, Manion, & Morrison, 2007). The questions were specific but many times the sequence of these questions was modified or added/removed depending on how the conversation evolved. The interviews were recorded by recording in all cases. The interviewer asked the students to express their thoughts and feelings about the future practical application of their theoretical training in their working environment, as well as finding ways of linking theory to practice in the workplace. Subsequently, the

views from the discussion with the students are presented as they talk with contemporary theoretical reflection.

#### 4. THE EXPERIENCE OF PRACTICE

The benefits to internships for the future educators are known and varied. Additionally, these expectations that have to do with the ability to be approaching an educational condition from a variety of visual materials, to cooperate to test a solution, while simultaneously they are able to wonder for educational values incorporated in the chosen solution. Taking this further in the future, educators will be able to investigate and assess the education act, taking advantage of data in perspective improvement of the act (Avgitidou et al., 2016). We also note that it is important to establish a stable link between the Department and the potential of the students with the labor market, the specialization and further development of the professional interests of the students, the formation of professional consciousness and the reinforcement of the prospects of professional rehabilitation of the students. During the practice, students' obligations are proposed to be the same as the employees' obligations and to enjoy equal treatment with them in order to acquire professional consciousness. How do students treat this prospect? The introductory questions of the interview, students' response was distinguished by intense anxiety to the students and this was apparent from their response. The participants were stressed about the way in which theory was applied in practice, but also about the framework of cooperation they were called upon to create with their colleagues.

*"There was anxiety about what it would be like if I could cope with all this, that is, how it would be, how I could do what I know. Not just for my knowledge. Everything to me is to have good cooperation. "*

To a certain extent, it seems that internship arranges these emotions or puts them in a more realistic context, as it contributes to strengthening, extending and qualitatively upgrading student education by linking it to research-action action (Sidiropoulou & Tsaoula, 2008). The necessary day-to-day contact with the workplace enables students to better assimilate the theoretical knowledge through the process of practicing and to acquire new knowledge and experience in their cognitive subject.

*"Whatever will be, will be. You do not enter childhood and you know everything, you are discussing them, tell them what you are afraid of and they will help you".*

By aiming at developing and highlighting students' skills and broadening their interest and skills in the professional field, the process contributes to a smoother transition of students to the workplace. The result is to create the necessary contacts for future professional rehabilitation and development so that they can meet the requirements of the professional space. In order to respond positively, students look for ways of linking theory to practice in the workplace. Through the discussion,

experimentation, action proposals and the search for ways in notes and bibliographic sources are noted as the most important element.

"I was trying to win the favor of educators and children on the first day, and that was done with one action. Thus, unintentionally or deliberately, the pedagogic shareholders were also involved in the process and involved in this process. I tried in a variety of ways to integrate theory into class and activity, and when an incident occurred in the classroom, when I was allowed, I would say my opinion. "

Students' practical training complements their theoretical training and contributes to their subsequent successful professional careers. Students' reflection on the connection of their theoretical studies with their practical application leads to the search for a way for a more effective coupling of theory - act. In particular, through the practice, students will come into contact with the working reality of the sectors that they have chosen to study and apply theories and knowledge taught and acquired during their studies. Thus, they will be able to identify and formulate teaching objectives, select appropriate teaching methods, understand the cognitive needs of their students, and orient their activities accordingly (Bouglas, 2007).

Evaluating their experiences and talking about any difficulties faced by students, they agree that they include poor co-operation with colleagues, general conditions, lack of professional experience by themselves, and the sense of control of their work by pedagogical staff. Focusing on feelings about cooperation issues they say:

*"There have been times when I proposed things and my teachers dismissed them, so I could not work in the class as I wished. I felt angry because they did not let me try in new situations with the children. "And another: "Of course I have encountered difficulties, mainly with the teacher's different perception of the realization of my actions. Once I suggested to two-year-olds to tear newspapers using their hands and the educator rejected my suggestion because she believed that they were not neurologically it was not mature, as she had argued. "*

*"I thought that whenever I applied something, the educator would judge me, so I took a few initiatives."*

*Of particular interest are those about the "shortcomings" they experienced in relation to professional experience.*

*"Certainly, and I found it difficult to apply theory to the act, since we were still infinite. I felt uncomfortable ... There were moments when the child was angry and I did not know how to handle it. "*

*"I felt low self-confidence because I did not have experience and I thought I could not have the primary role in a classroom."*

*"Yes, there have been difficult situations. For example, at a kindergarten I had gone, I was employing a child reading a fairy tale. At the same time, a baby with diagnosed autism came to us and started painting the wall with an indelible marker, and then the educator turned out irritated, telling me that I should watch the other*

*child and not read the tale. Okay, I know the kid had some difficulty and I had to be there. You start saying that I made a mistake and I am not completely responsible for the fact ...Another incident, I remember how panicked I was left alone with the children. I did not know how to organize and impose".*

In the context of the internship, the student is required to demonstrate that he/she has the ability to use the knowledge and skills he/she has developed during his/her studies by learning to work and working methodically, using combinational thinking to complete his /her work. However, when the lack of experience is combined with restrictions or conditions that students cannot manage with ease their self-confidence seems to be hit.

*"Too many times I challenged myself to use my knowledge and apply theory and at the same time myself and said I do not do this profession. This made me feel the bad conditions of the class and the lack of experience. When you have 15-20 children and one excites the other"*

*"Due to the lack of appropriate material, I experienced difficulties in conducting actions".*

But this fact, the swing of professional self-confidence, is not necessarily negative. Students who felt their abilities were not sufficient, very often, they were entering the process of reflection. For Dewey, reflection involves several phases, such as that of doubt and the hesitation that occurs when man is faced with dilemmas. These dilemmas trigger the thought, which will lead the individual to seek and explore the solution of dilemmas (Giddens, 2001). In these first experiences of coupling theory in practice, reflection brings a balance between experience and knowledge through their correlation by offering students the ability to process their personal experiences.

## 5. EXPECTATIONS FOR EFFECTIVE ADVISORY GUIDANCE

In this context, the role of the supervisor is understood in a context of broader mentoring practice (Mentoring) (Salvaras, 2013). In the Mentoring process, a group of students is practises in a specific learning environment where taught professors undertake multi-role consultations, supervising all internship of students (Fridaki, 2013). The teachings are subject to observation by the students. The supervisors organize the individual teachings of the trainees by conceding teaching time, observing, discussing and evaluating their efforts. In this context, the theoretical knowledge interferes with the act as the student is concerned about teaching and learning and re-examines his / her perceptions, but also the supervisor himself seeks alternatives with the student and broadens his / her view (Avgitidou et al., 2016). This direction creates a different "culture" in schools, participatory and oriented towards improving teaching and learning for all.

A central face within the internship of mentoring is that of the mentor-supervisor. The work of which includes: psychological support, professional

development assistance and the provision of a role model (Salvaras, 2013). It is a person professionally experienced and scientifically trained in a specific area or field, who guides the student so that he can enter professionally in field of activity. For students, the supervisor can be a source of information on teaching, a source of advice and feedback on their internship, and the main trusted advisor at all times (Salvaras, 2013).

The supervisor acts as a coach, animator and promoter (Beladakis, 2007). He cultivates trainees' feelings of acceptance of innovation (Theofilis, 2012), encourages, supports and facilitates learners in the implementation of innovation, discusses the progress of implementation, provides solutions to the problems that arise and fosters the spirit of cooperation. Supervisors also emphasize the professional development of prospective teachers by providing them with guidance, support, encouragement, advice and friendly support.

*"To be accessible, to be able to approach him, to have professional experience, to support you, to have good mood, to be an active listener".*

"To be communicative, keep it. Also, they can be next to the students and support them. To have transmissibility, to have special knowledge and experience. "

During the internship, the supervisor's multidimensional role includes assisting students to make the best possible use of their time in the field and to combine theory and practice effectively.

*"In order to feel secure I needed a supervisor, I also had to solve the questions I had for my work, but I also needed someone to talk about my concerns that were being created at kindergarten."*

It is innovation, pedagogical and educational and secondly organizational (Gianakaki, 2005). Through this process the student additionally learns to respond to a range of formal, service, extra-curricular tasks and knows closely how to organize, operate and manage an educational unit.

Correspondingly, the benefits are equally important for supervisors and this fact is recognized by both themselves and by the students. In response to a question about supervisor characteristics that make supervision effective, a student notes the following:

*"Active listening, willingness to answer questions, to be cooperative and to have in mind that he himself is assisted by the student."*

Through colleagues with colleagues, future colleagues, they are making thoughts again and again doing many flashbacks (Schön, 1994) the existing perceptions of teaching and learning consequently these gradually being transformed into a transformation of previous reference frameworks to make them more thoughtful, inclusive and emotionally suited to change (Mezirow, 2000). The presence of students is an incentive to use new teaching methods. Thus, the training process of future teachers renews the teaching practice (Bova & Phillips, 1984 · Simpson et al., 2007 · Mamoura, 2013).

## 6. CONTRIBUTION OF THE SUPERVISOR TO THE FIRST PROFESSIONAL EXPERIENCE OF THE TRAINEES

Monitoring in the sense of more observation of the first pedagogical applications of the students by the supervisor, in itself, creates a special condition. The diagnostic nature of the tracking, because it is not threatening for the trainee, can particularly offer the emergence of strengths and the improvement of the student's weaknesses (Sergiovanni & Starratt, 2002).

Do students feel that they are supported by this relationship? In order to investigate whether the academic framework contributes to the support of trainees, we asked the following question: "Is there any person in your training framework who has supported you in your practical exercise and if so, how did you communicate?" The students reported the teacher supervisor as the support person, however expressing great dissatisfaction and complaints about the way, level, degree and frequency of communication.

*"I think the mediator between me and the educators was the supervisor. He was the one who would help overcome some of the issues I had with them. "*

Some of the students even described "insufficient" supervision, since the communication with the supervisor was incomplete, his directions difficult and unresponsive to the needs of the students. The deficiencies concerned the absence of bibliographic enrichment, and the scientifically documented pedagogical response to student questions resulting from inexperience.

*"He was inadequate because he did not come often. But also, I did not correctly understand what she was asking many times. Additionally, they did not see us in action with children. Several of them liked to come, explain the work and leave. "*

The support comes, in addition to the supervisor, from other directions. Students report as sources of support: educators, fellow students, department professors and teacher supervisors.

*"Although there was oversight but I had accepted it once in my presence at the kindergarten, personal support was not from any of the faculty, it was rare for the supervisor to come, because it was not possible to continue there. I believe that meetings should be more frequent, at least every two weeks, as I think that the supervisor with his presence will help us and us, but he will see if our work is done properly. Because if we know that there is no proper supervision, we are more relaxed, and if we keep in mind that supervision is more frequent, we will work more intensely. "*

## 7. DETERMINING EFFICIENCY OF THE SUPERVISOR

Undoubtedly, the supervisor's role is a demanding one, it is a "key role" in the practice of students. By codifying his work, we can say that it includes three main



elements: psychological support, professional development assistance and the provision of a role model (Bouglas, 2007). It is a person professionally experienced and scientifically trained in a specific area or field, who guides the student so that he can enter professionally in his field of activity professionally. The term mentor-supervisor was mainly related to the empirical knowledge of teachers. For students, the supervisor can be a source of information on teaching, a source of advice and feedback on their practice, and the main trusted advisor in every circumstance.

The supervisor is required to have the appropriate scientific background to be able to describe and discuss his teaching practice. He has to have a wide repertoire of available teaching practices, to be used on a case-by-case basis. He needs to be willing to analyze and evaluate his work as objectively as possible.

*"To be receptive to offering help, to have specialized scientific knowledge and to prepare the environment of the child who will accept us".*

In addition, it is necessary to have counseling skills to support adults but also to maintain a quality relationship with learners on a personal level. The supervisor is useful to be receptive to students' ideas and ideas and to have communication skills, discretion and empathy (Sidiropoulou & Tsaoula, 2008).

*"To understand, to listen, to be supportive, to explain, not to be demanding and absolute, to have scientific knowledge and empathy because he had once been in our position."*

Still, it must be able to set achievable goals for the professional development of students. The supervisor is active in a context that includes both the school and the university department not only as a role-holder, but also as a person with his / her own traits and personality.

On an institutional level, its actions with the students are related to the teaching of the students in the classroom and the collaboration with them for the planning and organization of the class. What is more, the careful observation of its conduct and its feedback after its completion and, secondly, the development of the (cognitive, professional, etc.) as well as their support for the problems and difficulties that arise and the informal assessment of their progress.

On a personal level, the supervisor may initially help students cope with the "shock of the act", which implies the application in the practice of the theory taught, offering emotional support. It develops with the students a professional friendship, which is based on honesty, trust and mutual respect, in order to meet the goals and rules that have been set. The quality of interpersonal relationships between the supervisor and apprentice students is the key to successful collaboration, the promotion of learning and the achievement of the goals of the internship (Bouglas, 2007).

In order to successfully complete the internship of the students, it is essential that the supervisor cooperates with the head of the university department and his colleagues (Sidiropoulou & Tsaoula, 2008). In the first case it is necessary to create

channels of essential communication, the establishment of constructive cooperation and a coherent view of the precise definition of their role. In the second case, within the cooperation culture (Maurogiorgos, n.d) supervisors exchange ideas, coordinate their actions, collectively deal with common problems, process and prioritize appropriate solutions and become more efficient in the practice of students. Why, however, are students looking for communication with the supervisor?

*"Because the supervisor is the person who helps you solve your questions and is the person who knows how you should be in your first steps. He has more knowledge in pedagogic and psychological issues than ourselves, so he can direct us and as he sees and observes you helps you cope with a situation and evolve. "*

*"I remember once, I proposed actions in my practice and the educator corrected me continuously, but putting me in a difficult position as if controlling my knowledge. I had reported this incident to the supervisor and he had helped me to interpret the pedagogue's behavior after having discussed with the educator. "*

Discussing about the reasons of communication, we conclude that they primarily deal with regard to clarifications on the work and monitoring of pedagogical activities at the kindergarten. There is a need for the supervisor to act as a communicator for issues that students feel to be lagging behind because of inexperience, hesitation or lack of documented pedagogical views. Another part that the supervisor seeks is related to regulatory issues of working hours, attendance and general student obligations at the place of practicing the practical part of their studies.

With their specialized knowledge and experience, supervisors are the driving force behind the practical training of students. At all stages of its implementation, they formally and informally evaluate and submit improvement and review proposals. In this way, the application of innovation becomes a process of interaction, dialogue and feedback among those involved in that framework (Loizidou, 2012). The presence of the supervisor in the room causes a range of conflicting emotions from the most positive to the most dysfunctional depending on the supervisor. The students feel safe and relieved by their presence in the room and sometimes stress, the fear of being judged, even to the point of questioning their own abilities. It is worth looking at the feelings and thoughts experienced by the students at the times when the supervisor was present in their pedagogical activities

*"I was anxious, because if the supervisor that would come once would see that I did not participate, they would negate all the effort and work that I had done on previous occasions. Their view wouldn't reflect reality".*

*"When the supervisor was there, I was relaxed. (...) In general, I felt comfortable being comprehended in whatever problem that I was discussing. We used to find solutions, I did indeed feel stressed that I was being watched inside the classroom and that's why my performance didn't correspond to reality."*

*"It depends on the supervisor. Each of them conjured different emotions. On the one hand, I felt safe knowing that there's someone that could answer my questions,*

*but on the other hand I felt a little anxious about the situation at hand in which they'd happen to be present. It's just that you can always get stressed when you feel that you're being watched".*

Apart from the positive and negative feelings that were experienced by the people involved in the supervision, it is particularly interesting to see if they consider whether the supervision serves a role as a practice and the reasons why it does.

*"I believe that supervision is very important. That's because the supervisor is there when you're still a student, you haven't graduated yet, you haven't had any experience and you struggle without knowing if what you're doing is right or wrong. They serve as a reference point to help you adapt and evolve, and I wish there was a supervisor throughout my studies to watch my progression".*

*"I believe that the support from a person from the faculty is necessary because, with their knowledge and insight of my ability, they would help me put theory into practice, while I think that this is impossible. In the meantime, they'd explain with their expertise why something I did wasn't successful".*

Speaking about the positive results of the supervision, we shouldn't omit a field of particular gravity where its beneficial effects are noted. Supervisors act as intermediaries and agencies of prototype experimental ideas that diffuse in kindergartens. Thus, through the network of school units involved, there is an innovation upgrade (Ifanti & Kiriakopoulou, 2011). An "internal networking" (Siakoveli, 2011) is being developed between supervisors - students with an exchange of views and experiences, joint solutions to emerging problems and the submission of improvement proposals for the promotion of the results that were gained from the application of these innovations. The conscientious and consistent exercise of the role of the supervisors prevents innovation from turning into a formalistic process and makes it an essential means of improving the school unit (Mauroskoufis, 2002).

## 8. DRAWING FROM EXPERIENCE

A conversation between the views of the students directly involved and the theoretical argument on mentoring was attempted. Through the students' speech, there were highlights of the communication of the practice with the supervision. Optics that are complemented, amplified, expanded, but also refuted by synthesizing a large part of the mosaic of this institution are detected (Sidiropoulou & Tsaoula, 2008). The fact that the discussion that takes place on these perspectives stems from the students' direct personal empirical experience is of particular importance. The discussion highlights the effectiveness of supervision, students' expectations of a good guidance, their career in vocational training, the search for modern innovative pedagogical approaches and the role of supervisor in this context. It becomes apparent that supervision is considered necessary by the students throughout the course and process of pedagogical efforts and experiments carried out, provided that the way, the form,

the frequency and the characteristics of the person exercising its role are deemed appropriate and sufficient.

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## US EMPLOYMENT LAWS IN A MULTI-NATIONAL ENVIRONMENT: A COMPREHENSIVE ANALYSIS

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### ABSTRACT

*The question of whether U.S. employment discrimination laws apply to international employers is complex and involves multiple sources of legal authority including US statutes, international treaties, and the laws of non-American host countries (Posthuma, Roehling & Campion, 2006). Ryan, Weichmann & Hemingway (2003) noted that knowledge of legal issues is important when designing all types of best practices for global organizations. Therefore, legal expertise is an important part of professional practice of human resource management (Blakeney, Broenen, Dyck, Fran, Glenn, Johnson, & Mayo, 2002). This paper aims at identifying the importance of knowledge of the application of employment law, by investigating its international application, and examining guidelines for avoiding unlawful practices. Employment law practices in certain counties (Asia, UK, EU) are provided as an example.*

**Keywords:** ADA; Civil Rights Act; International Labor Organization; Employment Equality Directive; Racial Equality Directive.

### 1. INTRODUCTION

The development of the legal environment creates a significant and increasing challenge as businesses continue to expand their global operations (Brown, 1995; Savage & Wenner, 2001; Schuler, Dowling, & De Cieri, 1993). Unfortunately, the determination of whether U.S. discrimination laws apply to international employers is a multifaceted question. Multiple sources of legal authority including U.S. statutes, international treaties, and laws of non-American host countries interact to provide the controlling law. Further, the application of the law often requires the resolution of ambiguous factual issues. Moreover, in some situations where U.S. laws do apply, there may be legal defenses arising from international law justifying employment decisions that would normally be prohibited (Posthuma, Roehling & Campion, 2006). Before sending employees to the US, it is critical for foreign employers to understand the basic requirements of US wage and hour law to avoid unintentional but costly violations of that law (Woodford & Maes, 2002).

Companies doing business internationally understand that they will be governed by the laws of each of the country in which they operate. Just as an American tourist driving his rented motor scooter through Hamilton, Bermuda, must stay on the left side of the road, an American company operating in Bermuda must

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comply with that island's laws and regulations governing business (McPhillips, 2003). One of the hardships that American multinational companies often do encounter is the cost of complying with host-country labor laws and regulations. For example, the social-security costs imposed on companies employing German workers, including mandatory pension plans, can represent an additional burden of twenty percent (20%) or more on top of each employee's cash wages (with each employee matching that amount through payroll deductions) (McPhillips, 2003). Moreover, it can be extremely difficult to terminate employees under the laws of certain countries (such as Germany) without substantial advance notice or the payment of an expensive severance package. The American multinational company must observe such local labor laws while maintaining compliance with various U.S. employment laws that follow them abroad (McPhillips, 2003).

Whereas US jurisprudence occupies a hallowed status in circumscribing the relationship between American employers and employees, derivatives of other institutional systems such as religious canons and cultural customs are central forces in directing acceptable employment practices in other countries (Canty, Mohamed, & Robinson, 1996). Yet, as employers continue to cross national borders and as they open their organizations to employees from different countries, it is clear that the challenges of creating workplaces that are both legal and fair to all individuals will continue. It is incumbent upon employers, both American and foreign, to become familiar with employment and labor laws, as well as customs and traditions, of the countries in which they operate their businesses (Domagalski, 2008).

## 2. LITERATURE REVIEW

In 1964, Congress enacted the Civil Rights Act of 1964 which provided specific protection of individual rights in the workplace. Title VII of the act forbids an employer from discriminating on the basis of an employee's race, religion, gender, or national origin (Feldes, Robinson, & Fink, 1992). The primary objective of this legislation was to achieve certain national socio-cultural objectives in the work environment. While bearing this in mind, organizations internationalizing successfully are equally aware of the demands of the host country's legal and cultural practices (Feldes, Robinson, & Fink, 1992). The potential for conflict arises when the laws of many host countries do not provide the same level of protection for employees as found in Title VII. Furthermore, accepted business practices in some countries may even be contrary to the values of US business and society (Feldes, Robinson, & Fink, 1992).

When Congress ratified the Civil Rights Act of 1991, it amended Title VII of the 1964 Civil Rights Act and the 1990 Americans with Disabilities Act, to extent employment discrimination protection to American citizens employed abroad by US companies and by foreign corporations controlled by a US employer (Domagalski,



2008). The Age Discrimination in Employment Act also granted extraterritorial coverage in 1984 to US citizens over the age of 40 who are employed abroad for a US employer. The combined effect of these laws was to proscribe discriminatory conduct toward US citizens working overseas (Domagalski, 2008). The amendment in the 1991 Civil Rights Act has its genesis in a Supreme Court ruling brought by the Equal Employment Opportunity Commission (EEOC) against the Arabian American Oil Company during which the Court ruled that federal antidiscrimination law did not apply extraterritorial (Domagalski, 2008). As a response to the Supreme Court findings over the years, the Congress included provisions addressing the issues of extraterritorial protection to American citizens. The bill was passed and signed into law in November 21, 1991. Both inclusionary and exclusionary provisions were incorporated into the act; the overseas extension of civil rights protections is not, however, universal or automatic. If a conflict between US employment law and the host country's employment law arises in an overseas operation, then the US-based employer is expected to comply with the host country's law (Feldes, Robinson, & Fink, 1992).

Additionally, the three major employment discrimination statutes in the US are the Age Discrimination Act of 1967 (ADEA), Title VII of the Civil Rights Act of 1964 (amended in 1991), and the Americans with Disabilities Act of 1990 (ADA); any other employment laws are typically not applied outside the US (Posthuma, Roehling & Campion, 2006). The ADEA prohibits employers from discriminating against individuals 40 and over based on their age. In 1984, Congress amended the ADEA to extend coverage to US citizens working for US corporations outside the US. The amendments also extended coverage to corporations controlled by US firms, but they did not require employers to comply with the ADEA if it would violate foreign laws (Posthuma, Roehling & Campion, 2006). Title VII prohibits employers from discriminating based on race, sex, religion, color, or national origin in hiring, firing, or other terms and conditions of employment. The ADA prohibits employers from discriminating against qualified individuals because of their disabilities and also requires employers to provide a reasonable accommodation for employees when it is not an undue hardship for the employer (Posthuma, Roehling & Campion, 2006).

In 1991 the US Supreme Court ruled that Title VII did not apply to employees who are US citizens working outside the US, but Congress amended both Title VII and the ADA to provide that these statutes do apply to US citizens working outside the US for US employers (Posthuma, Roehling & Campion, 2006). These amendments were estimated to affect more than 2,000 employers operating outside the US (Starr, 1996). However, Title VII and the ADA do not apply to foreign corporation unless a US firm controls the foreign corporation. In addition, the 1991 amendments prohibit applying these statutes to situations that would require the employer to violate the laws of the foreign country (Posthuma, Roehling & Campion, 2006).

However, if the host country has no employment laws which explicitly ban the employment of certain minorities, followers of particular religions, members of specific nationalities, or women, then the employer is expected to ensure the US mandated equal employment opportunities of its US employees (Feldes, Robinson, & Fink, 1992). In other words, US citizens working for a US owned or controlled corporation's foreign operations are entitled to the same employment rights that they would enjoy had they been working at a domestic site belonging to that employee – unless these rights are prohibited by the laws of the host country (Feldes, Robinson, & Fink, 1992).

Until the passage of the Civil Rights Act of 1991, US corporations had no legal responsibility to follow the anti-discrimination tenets of Title VII in their foreign plants and subsidiaries. With its passage however, US corporations now may be sued by disgruntled employees in the US courts as a result of alleged bias in treatment, promotion, job assignment, or other conditions of employment in a non-territorial corporate facility (Feldes, Robinson, & Fink, 1992). The laws of the host nation will act as constraints on the legal protection possible to alleged victims of discrimination rather than as the only avenue of legal resource available to them as in the past (Feldes, Robinson, & Fink, 1992).

### 3. LEGISLATION IN ASIA, UK AND EU

As the European Economic Community (EEC) morphed into the European Community (EC) and now the European Union (EU), its role and influence in labor law and employment policy have expanded tremendously. The main pieces of labor-related EEC legislation in the 1970s required the member states to modify their domestic law and practice only in a few specific areas: free movement of workers within the EEC, preservation of collective and individual labor contracts during corporate transformations and successor ships, protection of workers' wages during employer bankruptcies, mass reductions in force, sex discrimination, and equal pay (Weiss, 2010). Today, each of those bodies of EU law has been strengthened through amendment and updating. From the 1990s onwards, new secondary legislation has been adopted covering areas ranging from workers' participation in strategic decision-making at both the workplace and enterprise level, the organization and limitation of working time, fixed-term employment, part-time employment, temporary employment, child labor, and occupational safety and health for young workers (Weiss, 2010). What started out under the EEC treaties as free movement of workers has morphed in more recent EU treaties into free movement of EU citizens. Secondary legislation regarding intra-EU mobility of workers, their rights to equal treatment by member state nationals, handling of accumulated health care and retirement benefits under the Social Security and employee benefit schemes, as well as individual labor

contract enforcement rights all have been strengthened in the last ten or fifteen years (Weiss, 2010).

European Union (EU) member states are implementing the Racial Equality Directive and the Employment Equality Directive to establish a general framework for equal treatment in employment and occupation (Lowe, 2008). Member states are charged with adopting legislation to prevent and remedy discrimination on the basis of, for example, age, gender, and disability, and are toiling with implementing protections against "lifestyle" discrimination, sexual orientation discrimination, among other concerns. These protections generally address both direct discrimination (disparate treatment) and indirect discrimination (similar to disparate impact analysis in the U.S.) (Lowe, 2008). In some cases, foreign law job protections may be more extensive than those provided under U.S. law. For example, the 2007 codification of the Labor Contract Law of the People's Republic of China provides that employment contracts cannot be terminated if the employee has been working for the employer continuously for more than 15 years and is less than 5 years away from retirement. In the UK, unlike the U.S., age discrimination protection has been defined to protect both older and younger workers, i.e., it is illegal to discriminate against an employee because the employee is either perceived as too old or too young (Lowe, 2008). Germany imposes quotas requiring employers with at least 20 employees to ensure that at least 5% of the workforce is composed of disabled employees and provides for additional days off for disabled employees. Moreover, most jurisdictions outside the U.S. reject the notion of "at-will" employment and have broad prohibitions against "unjust dismissal" (Lowe, 2008).

Recent developments in the employment discrimination laws of the European Union (EU) member states and the United Kingdom (UK) in particular, have highlighted the need for United States multinational corporations to be increasingly attentive to the rapidly developing labor and employment laws abroad. Such overseas legal developments will affect multinational corporations in areas including local compliance, global human resources, mergers and acquisitions, employee benefits, and even advertising (Berkowitz & Jackson, 2008).

On December 2, 2000, the Employment Equality Directive became effective in the EU, establishing minimum requirements to combat employment discrimination and requiring member states to legislate toward equal treatment in the areas of disability, religion, sexual orientation, and age (Berkowitz & Jackson, 2008). The Directive covers direct and indirect employment discrimination, applies to the public and private sectors, and encompasses working conditions, access to employment and vocational training, and membership in workers' or employment organizations. Most of the 27 EU member states had three years from the Directive's passage to legislate accordingly in the areas of religion and sexual orientation, and additional three years to legislate in the areas of age and disability (Berkowitz & Jackson, 2008).

The Employment Equality (Age) Regulations 2006 were implemented in the UK to comply with the legislative requirements for age discrimination of the European Employment Directive. The regulations became effective in the UK on October 1<sup>st</sup>, 2006, and cover employees of all ages working in the UK, including foreign employees (Berkowitz & Jackson, 2008). These regulations delineate a national default retirement age of 65 and the rights of employees to work beyond that age. They also prohibit discrimination, harassment, and retaliation by employers of employees on the basis of age (Berkowitz & Jackson, 2008). Different from laws in the United States, the focus is on prohibiting discrimination against older or younger employees; thus, the focus is not primarily on protecting older workers from age discrimination. Employers are prohibited from directly or indirectly discriminating against employees on the basis of age, unless the discrimination can be objectively justified by the employer (Berkowitz & Jackson, 2008).

These recent EU developments highlight the need for United States multinational corporations to be increasingly attentive of the rapidly developing labor and employment laws abroad. Indeed, further east, Japan and China have recently amended their laws to address employment discrimination (Berkowitz & Jackson, 2008). Japan's Employment Promotion Law addresses employment discrimination based on age in recruiting and hiring (and covers employees old and young, foreign and domestic). China's Employment Promotion Law addresses employment discrimination based on ethnicity, race, gender, disability, and religious belief (and gives employees, for the first time, the right to sue employers for discrimination) (Berkowitz & Jackson, 2008). United States multinational corporations need to understand the potential ramifications of noncompliance with overseas labor and employment laws, including the employment discrimination laws discussed above. Employers should review their recruiting, hiring, training, promotion, harassment, and benefits policies for potential implications of such discrimination laws. Multinationals also need to be increasingly vigilant in following the developing labor and employment laws abroad, as this area of law is changing rapidly, and ignorance of the requirements is not an acceptable alternative (Berkowitz & Jackson, 2008).

The U.S. Supreme Court has held that the Congress has the authority to regulate U.S. employers of U.S. citizens abroad, but that such coverage must be explicitly provided by statute (Lowe, 2008). As a result, other federal employment protections do not share the extraterritorial reach that Congress granted to Title VII, the ADA and the ADEA. For example, the National Labor Relations Act (NLRA), the Occupational Safety & Health Act (OSHA), and the Worker Adjustment & Retraining and Notification Act (WARN Act) apply only to workplaces within the U.S. and its possessions (Lowe, 2008). Similarly, only employees based in the U.S. are protected by the Family Medical Leave Act (FMLA), the Equal Pay Act (EPA), and the Fair Labor Standards Act (FLSA), none of which apply abroad. In addition, employees working outside the U.S. are not counted for determining coverage for purposes of the

FMLA, but are counted for determining whether an employer is subject to the WARN Act (Lowe, 2008).

Moreover, the majority of the countries in the Northeast and Southeast Asia are in the lower middle income and low income categories, with just one (Malaysia), in the upper middle category, and three (Singapore, South Korea, and Taiwan) in the high income category (Caraway, 2010). Economically, most of the countries in the region are relatively open to international trade and investment, but Laos, and North Korea are less integrated into the global political economy than the rest of the region. Politically, the region is divided equally between authoritarian regimes and procedural democracies; communist parties rule in most of the authoritarian regimes. Three countries (Cambodia, Malaysia, and Singapore) are semi-democracies that hold regular competitive elections in which the dominant party uses electoral rules, restrictions on civil liberties, and political intimidation to constrain the ability of opposition parties to effectively challenge it (Case, 1996; McCargo, 2005). The democracies in the region are relatively young, with the exception of the Philippines, which has experienced more years of democratic governance than any other country in the region. Wealth and democracy are correlated in the region, with most of the poor countries being authoritarian and the richer countries being democratic or semi-democratic (Caraway, 2010). Most East Asian governments have not embraced trade union. In contrast to Latin America where populist governments in some countries embraced union in inclusionary corporatist arrangements, most in East Asia sought to demobilize workers (Deyo, 1989, 2006; Koo, 2001). In the authoritarian countries ruled by communist parties, all unions are required to affiliate to the official union that is subordinate to the party (Gallagher, 2005; Clarke, Lee, & Chi, 2007; Taylor & Li, 2007).

China has also recently enacted a new law on labor contracts, which went into effect in 2008, the law places limits on the duration of fixed-term contracts (through regulating the total length of time and the number of renewals), outsourcing (by setting a minimum contract period of two years), and overtime (Wang, Appelbaum, Degiuli, & Lichtenstein, 2009). The new contract law represents an effort by the Chinese government to defuse social conflict in the export sector and to encourage investment in higher value-added industries (Caraway, 2010). The Chinese pushed ahead with the new contract law in spite of vocal protests by the Shanghai-based American Chamber of Commerce (Wang, Appelbaum, Degiuli, & Lichtenstein, 2009).

Moreover, several multilateral accords adopted by governments over the past 40 years have included ethical guidelines in addition to legislation in an attempt to influence the employment and trading practices of multinational companies (Frederick, 1991). Of the several accords described by researcher William C. Frederick, four specifically contain the relevant provisions (equality of pay, nondiscriminatory employment policies, and rights of the individual). These are the 1948 United Nations Universal Declaration of Human Rights which addressed

equality of pay, nondiscriminatory employment policies, and rights of the individual; the Helsinki Final Act of 1973 which addressed nondiscriminatory employment policies and the rights of the individual; OECD Guidelines for Multinational Enterprises in 1976 which covered nondiscriminatory employment policies; and the International Labor Office Tripartite Declaration of Principles Concerning Multinational Enterprises and Social Policy in 1977 which focused on equality of pay, nondiscriminatory employment policies, and rights of the individual (Feldes, Robinson, & Fink, 1992). These are summarized in the table below:

**TABLE 1: RIGHTS COVERED BY INTERNATIONAL ACCORDS**

Accord	Equality of Pay	Nondiscriminatory Employment Policies	Rights of Individuals
United Nations Universal Declaration of Human Rights, 1948	Yes	Yes	Yes
Helsinki Final Act, 1973			Yes
OECD Guidelines for Multinational Enterprises, 1976		Yes	
International Labor Office Tripartite Declaration of Principles Concerning Multinational Enterprises and Social Policy, 1977	Yes	Yes	Yes

**Source:** Feldes, P., Robinson, R.K., & Fink, R.L. (1992). *Equal Employment Responsibilities of Multinational Corporations*. Business Forum, ABI/INFORM Global, 18.

There is both domestic and international support for the furtherance of employment rights even without the specific sanction of law (Feldes, Robinson, & Fink, 1992). As members of the global community, multinational companies have a moral interest in the observance of those rules. It is also within the power of the countries involved to make these guidelines mandatory within their borders. In particular, if the US public perceives American multinational companies as not performing in the best interest of society and disregarding its standards, it may respond by seeking legislative redress (Feldes, Robinson, & Fink, 1992). Employment rights and the right to be free from discrimination in employment are increasingly recognized internationally as basic human rights. Where violation of employment rights is so egregious that it rises to the level of human rights abuses, claims can be brought in the International Criminal Court (Lowe, 2008).

Employment rights may also be protected through voluntary agreement. Historically, the principal source of employment protection for workers outside the U.S. has been legally binding labor agreements, and many countries still require an employer to have such an agreement, enforceable under local law, before an employer

is allowed to do business in that country (Lowe, 2008). In addition, multinational employers have been pressured to adopt corporate codes of conduct that guarantee a safe and healthy workplace, freedom from discrimination, minimum wages, reasonable limitations on work hours, the right to engage in collective bargaining, and prohibition on child labor or forced labor. These codes of conduct can be given greater reach if an employer insists that, as a condition of doing business, the suppliers and subcontractors also adhere to the same standards (Lowe, 2008).

Employers who meet minimum standards in nine areas, including employee health and safety, nondiscrimination, collective bargaining rights, and prohibition of forced labor and child labor, can be certified by Social Accountability International as meeting its Social Accountability 8000 standard (Lowe, 2008). However, adherence to corporate codes of conduct and most international labor standards is still entirely voluntary and extremely difficult to judicially enforce. Although a few cases have asserted that victimized workers are third-party beneficiaries entitled to sue for enforcement of the corporate codes of conduct, the case law in this area is still undeveloped (Lowe, 2008).

Labor-related international law, like international trade law, has been far from static during the past two and a half decades. Prior to the 1990s, international labor law encompassed little beyond International Labor Organization (ILO) conventions and recommendations, and certain provisions within the three "International Bill of Rights" human rights instruments: The Universal Declaration of Human Rights, the International Covenant on Civil and Political Rights, and the International Covenant on Economic, Social and Cultural Rights (Weiss, 2010). The ILO had adopted many international conventions and recommendations but relatively few from 1985 onwards. With the exception of the International Convention on the Worst Forms of Child Labor, recently adopted ILO Conventions have garnered relatively little ratification, which are required to render them binding on particular nation-states. Moreover, in the U.S. and many other countries, these instruments were often labeled "toothless" (Weiss, 2010).

In 1998, the ILO adopted the Declaration of Fundamental Principles and Rights at Work (Declaration), recognizing as universally binding upon ILO member countries four core labor rights: principles of freedom of association and collective bargaining, equality and non-discrimination, prohibition of child labor, and prohibition of forced labor (Weiss, 2010). Underestimated by many at the time of its adoption, the Declaration has been instrumental in shifting international labor law from a period of stagnation and near-futility to a wildly pluralistic period of enhanced effectiveness. The Declaration has fostered a wave of incorporation by reference of these core labor principles into a host of international public and private binding documents, often with stronger procedures for enforcement and remedies for violations than those of the ILO itself (Weiss, 2010).

#### 4. CONCLUSIONS

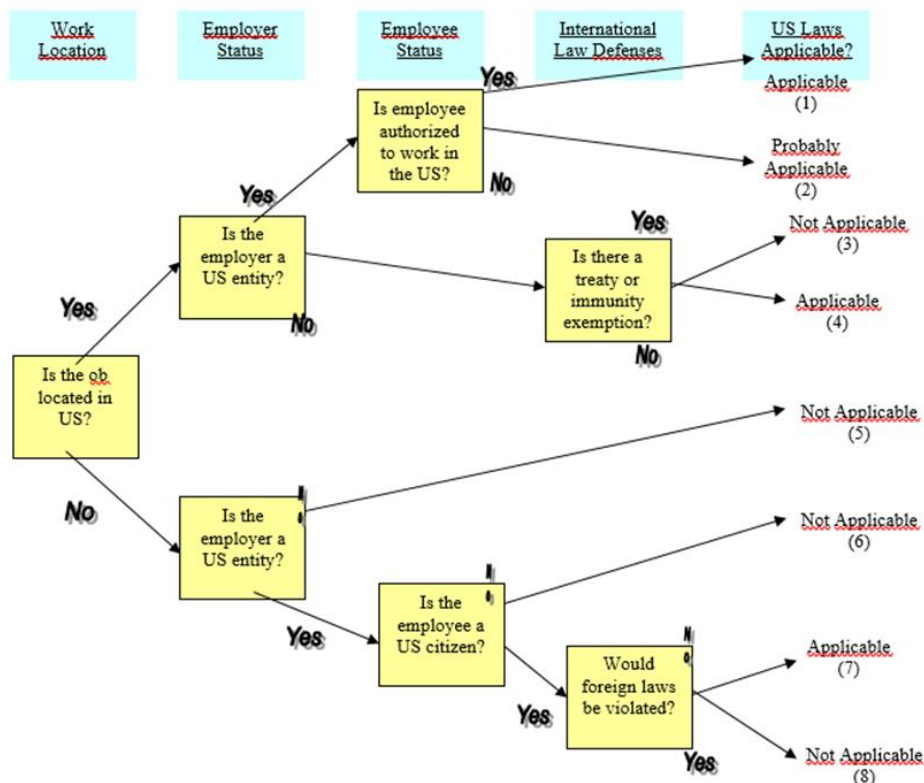
U.S. trade preference programs and trade agreements generally condition access to the U.S. market on a host country's compliance with "internationally recognized worker rights," including the right to organize collective bargaining, freedom of association, minimum wages and hours of work, and prohibitions on forced labor and child labor (Lowe, 2008). However, a private company's violation of these principles is actionable only if the host government fails to enforce these standards. In other words, the standards apply to governments, not directly to private actors. Moreover, private companies may be required to meet minimum labor and employment standards as a condition of obtaining financing for overseas projects. The U.S. Overseas Private Investment Corporation (OPIC) conditions financing for overseas projects on an employer's adherence to the same "internationally recognized worker rights". The World Bank's International Finance Corporation went further, adopting standards that require projects in which it invests to "base the employment relationship on the principle of equal opportunity and fair treatment." Americans working overseas on projects receiving funding from one of these sources can insist that the employer comply with these minimum standards; if the employer refuses, it risks losing the funding (Lowe, 2008).

Posthuma, Roehling & Campion (2006) have crafted eight guidelines emerging from several court cases regarding the Acts. In the process of formulating these guidelines, they have identified four factors that determine the applicability of US employment discrimination laws: (a) Location of the work (inside or outside the US), (b) Employer status (number of employees, home country), (c) Employee status (US citizen, authorized to work in the US), and (d) International law defenses (international treaties, foreign law defenses).

The complex interplay of these four factors is illustrated in a parsimonious algorithm model presented in the form of the decision tree in Figure 1:



**FIGURE 1: DECISION TREE (NUMBER IN PARENTHESIS MATCHES THE GUIDELINES BELOW)**



**Source:** Posthuma, A.R., Roehling, V.M. & Campion, A.M. (2006). *Applying US Employment Discrimination Laws to International Employers: Advice for Scientists and Practitioners. Personnel Psychology.*

According to (Posthuma, Roehling & Campion, 2006), the guidelines they specify when US Employment Discrimination Laws (Title VII, ADEA, ADA) apply to international employers are (according to the endpoints in the decision tree which corresponds to one of the eight guidelines presented below):

1. US employment discrimination laws apply to jobs located inside the US when the employer is a US entity and the employee is authorized to work in the US.
2. US employment discrimination laws apply to jobs located inside the US when the employer is a US entity and the employee is not a US citizen but is legally authorized to work in the US. Depending on jurisdictional issues, US laws may apply

to workers who are not authorized to work in the US, although the remedies they receive may be limited.

3. US employment discrimination laws do not apply to jobs located inside the US when the employer is a foreign entity exempted by a treaty, even though the employee is authorized to work in the US.

4. US employment discrimination laws apply to jobs located inside the US when the employer is a foreign entity not exempted by a treaty and the employee is authorized to work in the US.

5. US employment discrimination laws do not apply to jobs located outside the US when the employer is a foreign entity, even though the employee is a US citizen.

6. US employment discrimination laws do not apply to jobs located outside the US even if the employer is a US entity, if the employees are foreign citizens.

7. US employment discrimination laws apply to jobs located outside the US when the employer is a US entity and the employee is a US citizen, if compliance with the US laws would not violate foreign laws.

8. US employment discrimination laws do not apply to jobs located outside the US when the employer is a US entity and the employee is a US citizen, if compliance with US laws would violate foreign laws.

In many circumstances, international employers may influence whether US discrimination laws apply to their employment relationships through the policies they adopt, the practices they implement, and their conduct in dealing with employees (Posthuma, Roehling & Campion, 2006). Relative to this, organizationally sensible and responsible decisions require that other factors must be taken into account, including ethical issues, organizational values, and potential human resource management concerns (Roehling & Wright, 2004). Below, several of these factors are presented, providing guidance that will help managers contribute to international employer policies and practices that are both legally defensible and organizationally sensible:

- a) *Make consistency and organizational justice primary concerns*: consistent policies and practices provide employees a clearer sense of what they can expect and what is expected of them. Inconsistent policies may create mistrust and may contribute to invidious social comparisons and feelings of distributive injustice (Baron & Kreps, 1999).
- b) *Conduct professional job analyses that yield written job descriptions*: Job descriptions should include information on the location of an employee's work -an issue that may affect whether US discrimination laws apply-, descriptions of job responsibilities, etc.
- c) *Verify local 'legal concerns' that are offered as a basis for differentiating staffing practices*: Before adopting a local staffing practice that will be applied

- to US citizens working aboard, and potentially conflict with US employment laws, it is absolutely essential that the existence of a local law requiring the practice is verified; consult an attorney (Ryan *et al.*, 2003).
- d) *Design internationally-sensitive training*: Foreign managers going to the US should receive training that familiarizes them with US discrimination laws. Conversely, US managers going abroad should receive training that familiarizes them with the employment laws of their host country (Taylor & Eder, 2000).
  - e) *Consider the impact of organizational structure and job design on legal obligations*: The system and structure of an organization may be related to legal obligations in term of authority and decision making (who makes decisions). For example, international management systems (including compensation systems) are centralized to varying degrees (ethnocentric, polycentric, and geocentric) (Adler, 1983; Caligiuri & Stroh, 1995; Heenan & Perlmutter, 1979).
  - f) *Consider international legal implications in designing recruiting and selection procedures*: When establishing job requirements for employee selection systems, managers should know which laws apply so they can determine what criteria may be prohibited or required. For example, in the US age and national origin are prohibited forms of discrimination. Yes, when recruiting in the US for work in another country: age, citizenship, and immigration status may be job-related requirements.
  - g) *Recognize the potential value of written employment contracts*: From a legal perspective, written documentation provides evidence that is given great weight by the courts, and is often viewed as dispositive on the issue of what country's laws apply (Sabiru-Perez, 2000).

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## SURFACE INTERACTIONS AND CELLULAR UPTAKE OF METAL NANOPARTICLES IN PRIMARY LYMPHOCYTE CULTURES - BRIEF REPORT

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### ABSTRACT

*Gold and silver nanoparticles of 20, 40 and 100 nm in diameter were introduced into the culture medium of short-term lymphocyte cultures for studying the possibility to use them as carrier for therapeutic and immunomodulatory agents. Lymphocyte cultures from human volunteers and laboratory rats of Wistar strain were used. The ultrastructural analysis revealed that the nanoparticles congregated mostly in the extracellular space around the cell membrane without changes in the cell morphology being observed. Some methodological details associated with the use of suspension cultures are described. The results are discussed against the background of similar studies still few in the literature.*

**Keywords:** *Gold and Silver Nanoparticles; Short-Term Lymphocyte Cultures; Ultrastructure.*

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## 1. INTRODUCTION

Autoimmune thyroid diseases (AITD) are caused by interactions between genetic and environmental predisposing factors leading to autoimmune deterioration. During the last several years the available diagnostic and therapeutic methods as well as clinical consensus and guidelines have been updated, which helps practitioners to identify the proper management of the pathogenic mechanisms underlying AITD.

In the present work we share our experience in preparation and investigation of the nanoparticles interactions in short-term lymphocyte suspension cultures with emphasis on some difficulties we faced during the experiments. According to our research program concerning autoimmune diseases, an analysis of various nanotechnology products as potential carriers of drugs or immunomodulatory agents to different populations of lymphocytes, potentially responsible for autoimmune response was performed. T lymphocytes were used as cellular therapeutics in many diseases including autoimmunity and cancer. Our experiments were conducted with human and rat short-term lymphocyte cultures, with metal gold and silver nanoparticles introduced in the culture media. The preparations for electron-microscopic investigations were elaborated.

The aim of this study was to obtain ultrastructural evidence of lymphocyte T-cells and metal nanoparticles interactions, with respect to the available surface position or cell uptake.

### *1.1. Research Objectives*

- To analyse the interplay of gold or silver nanoparticles and T-cells in short-term lymphocyte cultures, obtained from peripheral blood samples of healthy patients and rats.
- To test systems for signs of toxicity or other pathological changes.

## 2. LITERATURE REVIEW

In the modern approaches in medicine the use of nanotechnology has started to gain strength and supporters. These researches give the opportunity to revolutionize the treatment of a large number of diseases and to point specific targets, minimizing injuries to the organism. Nanomedicine employs nanoparticles as drug delivering objects for direct treatment of particular types of cells on the basis of their engineering. Ensuring that healthy cells are maintained, nanoparticles are specifically loaded with agents directed to specific receptors of diseased cells as approach for attacking them. Nanotechnology and targeted drug therapies have already been used in



many diseases as the basis for new, more effective and selective drug delivery systems (Arap et al., 1998; Ediriwickrema and Saltzman, 2015; Rai et al., 2016; Vasir and Labhasetwar, 2005). These new drug therapeutic agents have already been shown to cause fewer side effects and be more effective than traditional therapies (Blanco et al., 2011; Gwinn and Vallyathan, 2006; Wiwanitkit et al., 2009; Yossifova et al., 2014). Nanomaterials can support treatment both in vivo and in vitro biomedical applications mainly due to their size - similar to most biological molecules and structures. The potential of nanomaterials has been detected and discussed as a valuable and diverse medico-technological approach towards a variety of diseases, which still have to be comprehensively studied (Balansky et al., 2013). Moreover their effectiveness, movement, and how well substances are being distributed or how they are metabolized can be traced and evaluated (Kato, 2011). One of the methods in use for visualization of nanoparticles is the transmission electron microscopy (TEM) (Asoro et al, 2013, Hannemann et al., 2006; Lovern et al., 2008; Zhou et al., 2012). According to data reported, among the metal nanoparticles, noble metal nanoparticles have demonstrated potential biomedical applications and showed role as novel antimicrobials (silver nanoparticles) and efficient drug carrier (gold nanoparticles) (Rai et al., 2016).

### 3. METHODOLOGY

#### *3.1. Experiment design*

Six male, two years old, clinically healthy Wistar rats were exsanguinated into heparinized tubes under anesthesia according to institutional guidelines and in accordance with the requirements of ARRIVE guidelines and the associated guidelines EU Directive 2010/63/EU for animal experiments regarding laboratory animals and animal welfare. Human venous blood was collected from two middle-aged healthy volunteers into standard heparinised tubes in the clinical laboratory of The 22<sup>nd</sup> diagnostic – consultative center, Sofia, Bulgaria. Lymphocytes were isolated from blood samples via Ficoll-Paque (Pharmacia, Uppsala, Sweden) and gradient centrifugation at 1850 rpm-1 for 40 minutes at 20°C. The resulting lymphocyte-mediated intermedial ring was aspirated with a cross-section needle syringe, washed twice with RPMI-1640 chilled media (4°C) and centrifuged at 1200 rpm -1 for 10 minutes at 4°C. Cells ( $5 \times 10^6$ ) from both species were seeded onto tissue culture flasks (Orange Scientific, Belgium). During the whole period of the experiment of 48h-72h the cells were cultivated in a humidified incubator containing 5% CO<sub>2</sub> at 37°C. Short-term lymphocyte cultures were incubated with a medium separately supplemented with citrate-stabilized metal nanoparticles: gold of 100 nm or 40 nm in diameter, or silver of 20 nm (0.1 mL standard suspension of respective nanoparticles/1.0 mL cell suspension) (Sigma Aldrich Chemie GmbH, Germany), after 24-36h of their initial placement for more 24-36h. Untreated lymphocytes were used as a negative control.

After the incubation for a predetermined time, the lymphocytes were prepared for TEM observations.

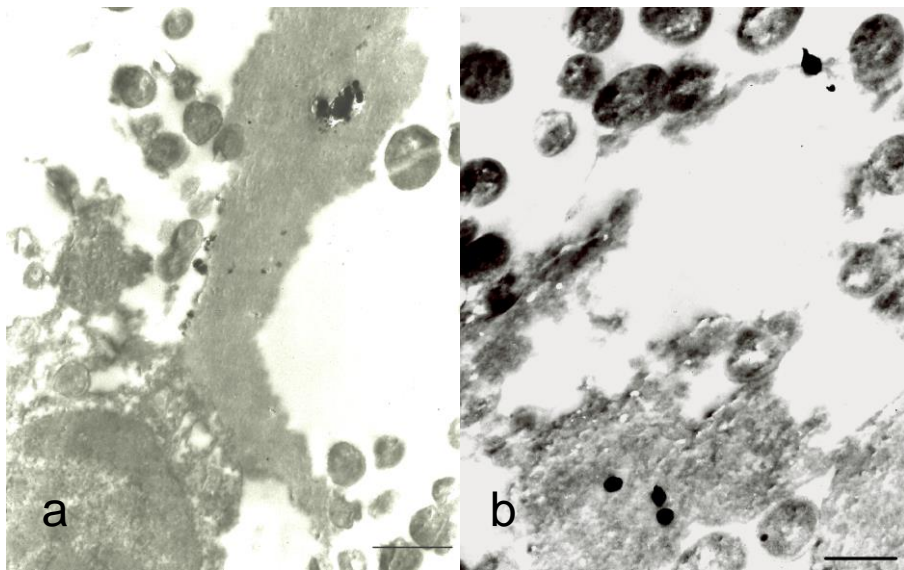
### *3.2. Transmission electron microscopy*

The cells were fixed for 120 min in 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.4 and after dehydration in ascending alcohol series, were embedded in Durcupan ACM /Fluka/. Ultrathin sections were stained with uranyl acetate and studied on a Jeol 1200 EX electron microscope and accelerating voltage of 80 kV.

## 4. FINDINGS

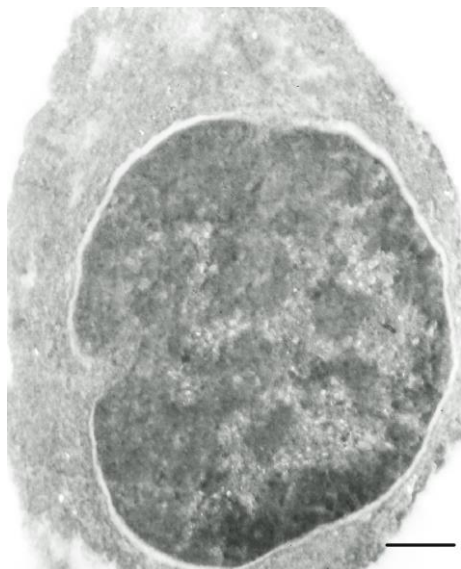
After 24 hours of exposure, the gold and silver nanoparticles congregated around lymphocytes and were not found within cells or in intracellular vacuoles due to certain cellular uptake- penetration, endocytosis, or semi-endocytosis. Their amount in the extracellular fluid was significant. The presence of the used nano-materials did not lead to any observable pathological change of the lymphocytes of the treated cultures and the cell morphology was comparable with those of the untreated controls (Figures 1a, 1b; 2). The metal nano-materials were piled tightly, in clusters or separately distributed throughout the intercellular space or near the plasma membrane of clearly visible lymphocytes with preserved structure.

**FIGURE 1:** 20NM SILVER NANOPARTICLES ON THE SUPERFICIES OF THE CELL MEMBRANE OF RAT LYMPHOCYTE



*a) Scale bar 500 nm; b) 100 nm gold nanoparticles on the superficies of the cell membrane of human lymphocyte.*

**FIGURE 2: CELL FROM CONTROL LYMPHOCYTE CULTURE**



*Scale bar 250 nm.*

### 3. CONCLUSIONS AND DISCUSSION

We assume that nanoparticles did not locate close to cell plasma lemma, due to their ability to move in the medium mimicking natural metabolism, adhesion or were homed specifically to cells via receptor-mediated endocytosis or phagocytosis found only in specialized cells. More likely, these results were due to some physical forces of attraction and surface interactions between the metal nanoparticles themselves or with the cells' superficial electrical charges. The knowledge of nanoparticles uptake is crucial for risk assessment and is essential in order to predict their behaviour based on their physical-chemical properties ( Kettler *et al.*, 2014). This study confirmed that the uptake depends strongly on size and electrical surface charges, either positive or negative, and the degree of homoaggregation (Kettler *et al.*, 2014). In our study we reported a disproportionate concentration of nano-material, which may be due to size surplus or not enough dilution of imported substrate, which could be more considerable than recommended. Another possible reason for this could be the non-efficient washing between the different steps, because of the suspension type of lymphocyte cultures. In those cultures, the washing between stages is definitely less operative compared to monolayer cultures.

The obtained results in this work present some specific observations on human and rat lymphocytes exposed to gold and silver nanoparticles by *in vitro* assay. In medicine, there is limited knowledge on the hematological effect of nanoparticle on blood cell types and such observations still need a detailed scientific verification. In our study the accumulation of the particles was exclusively in the intracellular space, around the cell membrane and no nanoparticles were found inside the cytoplasm. The interpretation for the absence of unique intracytoplasmic localization of nanomaterials in our study could not be the pore size in the lymphocyte membrane about 4 nm x 2.5 nm in diameter, which is much smaller than the diameter of used gold and silver nanoparticles. The direct penetration in the lymphocytes cytoplasm of gold nanoparticles, 9 nm in size, demonstrated and reported by Wiwanitkit *et al.* (2009;2009), was not due to mechanisms of phagocytosis and still the exact mechanism requires more studies according to the authors.

In modern researches, polymeric nanoparticles seem to be more suitable as nanocarriers for direct access and manipulation of antigen-specific T cells (Zupke, *et al.*, 2015).

However, in conclusion, our survey did not confirm lymphocyte uptake, release or toxicity of the applied gold and silver nanomaterial of 100, 40, or 20 nm in diameter. The actual direct effect of both types of nanoparticles, regardless of their size, was the observed non-specific superficial agglomeration on the cell membrane or in surrounding medium.

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## EVIDENCE OF PRODUCTIVE HHV-6 INFECTION IN AUTOIMMUNE THYROIDITIS PATIENTS' THYROID GLAND TISSUE SAMPLES

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### ABSTRACT

*The present work describes several cases of confirmed autoimmune thyroiditis (AIT) in two females and one male, patients with clinical diagnosis of Struma nodosa after long-term medical treatment following a total thyroidectomy. In the thyroid gland tissues of all three patients significant HHV-6 load (500> copies/10<sup>6</sup> cells) was accounted. (By means of transmission electron microscopy were observed HHV associated viral-like particles and characteristic feature of herpes virus infections - multivesicular bodies (MVBs)).*

**Keywords:** Autoimmune Thyroiditis; HHV-6 Load; Transmission Electron Microscopy.

### 1. INTRODUCTION

Autoimmune thyroid diseases (AITD) including Hashimoto's hypothyroid autoimmune thyroiditis (HT), hyperthyroid Graves disease (GD), and subtle subclinical thyroid dysfunctions are common autoimmune diseases, which might have

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no signs in their early development, but over some time it can progress and destroy the thyroid gland. Common complications of these diseases are hypo- or hyperthyroidism, blood pressure and reproductive problems, weight gain or loss, feeling tired or anxious, constipation, depression, general pains, etc which seriously decrease their quality of life. As a potential accompanying condition thyroid lymphoma may occur in some cases. AITD appears when T - lymphocytes mistakenly are prone to attack their own thyroid tissues with the help of antigen-presenting cells (APC)-professional APC or thyrocytes themselves, carrying specific antigens. Attempts in different directions try to investigate and to understand the origin of these processes and to develop specific aetiologically based therapeutic agents. Environmental, genetic and other endogenous factors could lead to formation of thyroid autoantigens and to provoke activation of autoreactive lymphocytes and plasma cells. Recently, more evidence has been collected where viral, and particularly human herpes virus infection can provoke the onset of autoimmune thyroid disease. We have been investigating this topic through the last several years and found productive HHV-6 infection in the glands of patients with autoimmune thyroid diseases.

The aim of this study was to assess ultrastructural investigation of AITD patients' thyroid tissues for additional evidence of active viral (HHV-6) presence in the thyroid gland tissues.

### *1.1. Research Objectives*

- To test thyroid gland tissue samples and peripheral blood samples of patients with confirm AITD diagnosis for HHV-6 sequences.
- To test patients' plasma samples for anti- HHV-6 specific IgG class antibodies.
- To find ultrastructural evidence for active HHV-6 infection.

## **2. LITERATURE REVIEW**

AITD are chronic inflammatory diseases of the thyroid gland with high health impact on the society and have been studied thoroughly (Kraiem *et al.* 1992; Hollowell *et al.* 2002; Yoo and Chung 2016).

The incidences of (AITD) seem to be increasing in recent times, according to conducted surveys all over the world in population after the age of 30 (Hollowell *et al.* 2002). HT has a distribution rate of 1-4%, affects 3-6/10000 population per year and is the second most common thyroid lesion diagnosed by fine needle aspiration after endemic goiter in iodine (I<sub>2</sub>) deficient areas (Kumar *et al.* 2002; Kocjan 2006, Ekambaram *et al.* 2010).

During the interaction between lymphocytes and thyroid cells are activated apoptotic pathways, through surface receptors, sensitive to certain cytokines



(produced locally by infiltrating T lymphocytes), which may play an important role for deregulation of apoptosis and the clinical expression especially in pathogenesis of HT (Fountoulakis *et al.* 2004).

Recently, more studies focused on Human herpesvirus 6 (HHV-6A and HHV-6B) infection were held, considering it as possible co- etiological factor for triggering AITD primarily due to its immunomodulating properties and the detection of elevated viral DNA copies in patients compared to controls (Prummel *et al.* 2004; Leite *et al.* 2008; Thomas *et al.* 2008; Desailoud and Hober 2009; Caselli *et al.* 2012). HHV-6 infection usually occurs in childhood and afterwards establishes lifelong persistence which often stays in latent stage. When triggers for reactivation occur, such as injury, physical, emotional stress, hormonal imbalances (Isegawa *et al.* 2010), or other specific factors, herpes viruses can pass into their active stage (Tang *et al.* 2010) and eventually surge as triggers initiating immunomodulating processes and self-reactivity (Dagna *et al.* 2013). The mechanisms of autoimmunity in AITD like Hashimoto's autoimmune thyroiditis, associated with active herpes virus infection, have not been investigated in detail yet. There are proposed different mechanisms of the viral involvement in AITD, such as: molecular mimicry, protein change and formation of cryptic self-epitopes, bystander activation (with or without epitope spreading), viral persistence (Vanderlugt and Miller 2002; Fujinami *et al.* 2006).

According to results reported by S. Benedetti (Benedetti 2012), HHV-6 is capable of long-term persistence in follicular cells, as shown by the persistence of viral DNA and latency-associated U94 gene. After cell separation, the authors established HHV-6 U96 positive epithelial cells virus load at mean value  $4.1 \times 10^4$  copies/ $\mu\text{g}$  DNA, while in non-epithelial (lymphocytes and monocytes)  $5.0 \times 10^2$  copies/ $\mu\text{g}$  DNA were reported. According to our previous results in blood samples of AITD patients, the HHV-6 viral load was detected only in one patient ( $46 \text{ viral copies}/1 \times 10^6 \text{ cells}$ ), while in all HHV-6 positive thyroid tissue samples the viral load was from 132 to 1620 viral copies/ $10^6$  cells. Substantial HHV-6 load in lymphocytes subpopulations was detected in NK and CD95<sup>+</sup> cells (Sultanova *et al.* 2016). The ultrastructural studies of viral particles characteristics for HHV-6 in human thyroid biopsies have not been proposed until now. Data of electron detection in other human materials is very limited (Roffman *et al.* 1990; Novoa *et al.* 1997; Randhawa *et al.* 1997; K hl *et al.* 2014). The acquired results and literature data had provoked an interest of ultrastructural investigations of thyroid tissue samples, tracing the eventual virus detection.

### 3. METHODOLOGY

In this study three patients were enrolled after total thyroidectomy. One female at the age of 41 with clinical diagnosis *Struma nodosa III euthyreoticum*, male at the age of 45 with clinical diagnosis *Struma nodosa III-IV thyreotoxicum* and female at

the age of 28 with clinical diagnosis *Struma nodosa IV thyreotoxicum*. To confirm the AITD diagnosis, patients were additionally tested for auto-antibodies against thyroid peroxidase (TPO), thyroglobulin (TG), and thyroid stimulating hormone receptor (TSHr), using commercial enzyme-linked immunosorbent assay (ELISA) kits (EUROIMMUN, Germany).

DNA from whole blood and thyroid gland tissues was extracted using the phenol-chloroform method. RNA from peripheral blood mononuclear cells (PBMC), thyroid gland tissue and control groups' autopsy specimens was extracted using TRI-reagent (Life Technologies, USA) in accordance with manufacturer's protocol, and cDNA synthesized using innuSCRIPT One Step RT-PCR SyGreen Kit (Analytik Jena, Germany). The quality of genomic DNA and synthesized cDNA, obtained from patients and autopsies, was determined by beta ( $\beta$ )-globin polymerase chain reaction (PCR) with appropriate primers (Van Damme *et al.* 1995).

Nested polymerase chain reaction (nPCR) was used to detect HHV-6 genomic sequence in 1  $\mu$ g of template DNA from whole blood and thyroid tissue (Secchiero *et al.* 1995). PCR was done as a two-step reaction with two sets of primers. Positive control (HHV-6 genomic DNA; Advanced Biotechnologies Inc, Columbia, MD, USA) and negative controls (DNA obtained from practically healthy HHV-6 negative blood donor and water controls) were included in each experiment.

For the approval of obtained PCR results, determination of HHV-6-specific IgG class antibodies in plasma was carried out using commercial ELISA kit (Panbio, Sinnamonn Park, QLD, Australia). The reaction results were calculated and analysis was performed out in accordance with the manufacturer's protocol.

HHV-6 specific mRNA (U79/80, U51 and U12) was detected using reverse transcription PCR (RT-PCR) in order to reveal active HHV-6 replication (Kondo *et al.* 2002). HHV-6 load in whole blood and tissue samples from patients with HHV-6 persistent infection was determined using HHV-6 Real-Time Alert Q-PCR kit (Sacace, Italy) and Applied Biosystems 7500 Real-time PCR System (Applied Biosystems, Carlsbad, CA, USA), in accordance with the manufacturer's recommendations. Collected data were processed and analyzed with specialized software ABI 7500 system. The data was calculated to get the viral copies /10<sup>6</sup> cells.

Light microscopic examinations were performed on epoxy semithin sections /thickness of 0.5-1 microns/ of Durcupan ACM Fluka resin embedded specimens cut by ultramicrotome Reichert-Jung Ultracut, Austria. Materials were stained with 1% toluidine blue in 1% sodium borate (Sigma Aldrich Chemie GmbH, Germany) (Richardson *et al.* 1960). Microphotographs were made on Leica DM 5000B, Wetzlar, Germany. For transmission electron microscopic analysis, thyroid tissues from patients were fixed with 4% phosphate buffered glutaraldehyde and postfixed in osmium tetroxide (0.5%) (Sigma Aldrich Chemie GmbH, Germany), dehydrated, and embedded into Durcupan ACM Fluka epoxy resin, according to standard procedure. Ultrathin sections /0.1 microns/ were made by ultramicrotome Reichert-Jung Ultracut,

Austria and double-stained with 2% uranyl acetate and 2% lead citrate (Sigma Aldrich Chemie GmbH, Germany), and viewed on a JEOL 1200 EX transmission electron microscope and accelerating voltage of 80 kV.

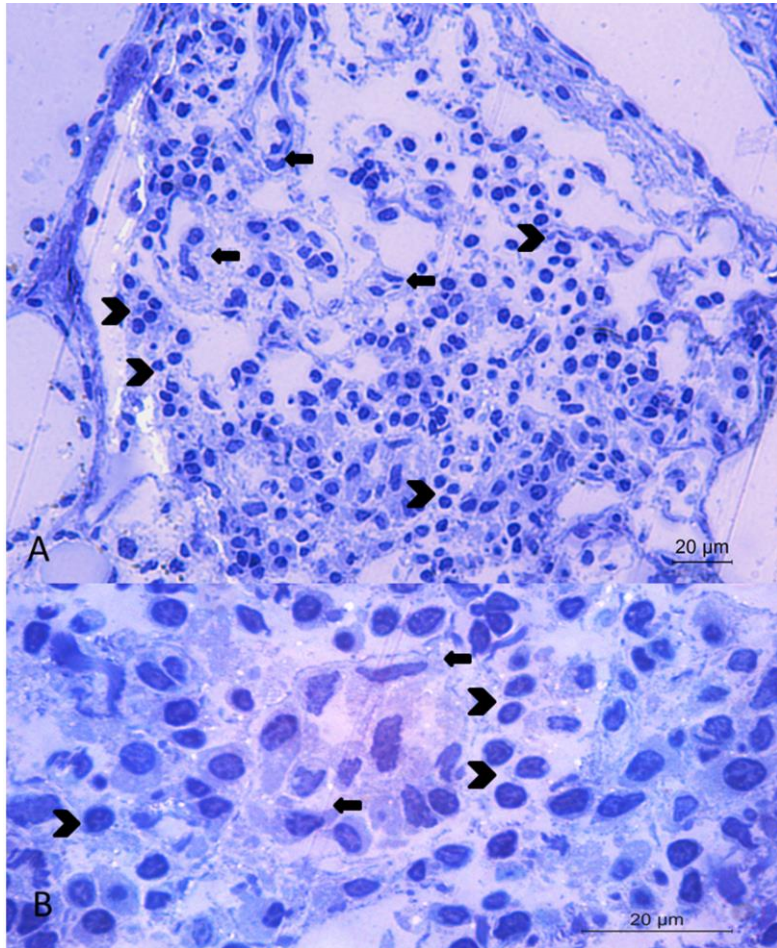
#### 4. FINDINGS

In all three patients elevated auto-antibodies against TPO, TG, and/or TSHr were detected. HHV-6 sequences were found only in patients' thyroid gland tissue samples, none of it was found in peripheral blood samples. Restriction analysis with *Hind*III endonuclease incubation shown HHV-6 B in all tested samples. Although, none of the blood samples revealed the presence of HHV-6 genomic sequence, all plasma samples were positive for anti- HHV-6 specific IgG class antibodies, using a qualitative ELISA test. All AIT patient thyroid gland tissue samples were positive for HHV-6 U79/80, U 51 and U12 mRNA. Using Real-Time polymerase chain reaction HHV-6 load was determined in patients' whole blood and thyroid gland tissue. In patients' blood the viral load was not detected in any DNA sample, while in all HHV-6 positive tissue samples viral load was detected (1306, 802 and 5923 viral copies/10<sup>6</sup> cells).

In all three cases the histologic appearance of thyroid follicles and colloid varied to some extent as a reflection of the secretory activity of thyroid glands. Variations in the follicular cell morphology and size were noticed-from large follicles and flattened thyroid epithelial cells at the periphery to smaller ones with less colloid and cuboidal epithelium in central parts of the gland. As a general finding we observed an abundant lymphocytic and plasma cell infiltration among follicular epithelial linings, rising to destruction and atrophy of thyroid follicles (fig. 1). In one case increased fibrosis was found and in two cases - secondary changes of necrosis and hemorrhages.

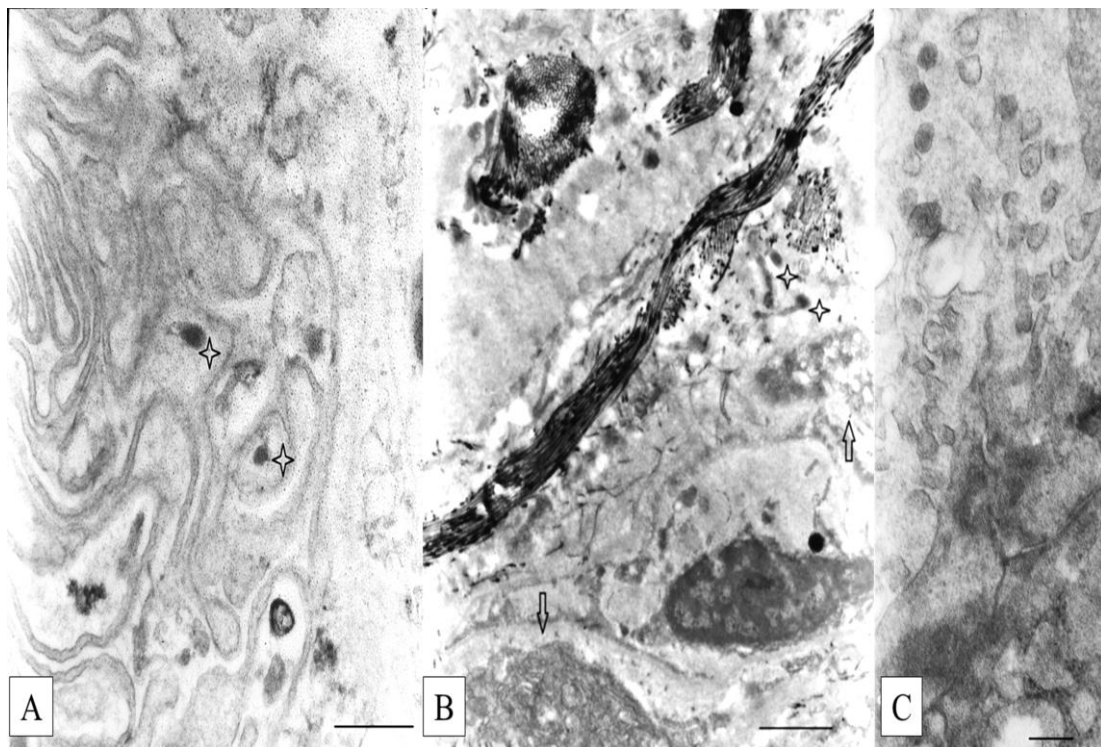
Ultra structurally, single or numerous viral-like particles and tegument-like materials were observed, consistent with morphological features of HHV-6 predominantly with intracellular distribution in the follicular subset of thyroid cells, rarely in the extracellular compartments (Fig. 2 A, B, C). The viral-like particles and tegument-like materials were present in the cytoplasm of infected cells, some in the cytosol in the Golgi apparatus (fig. 2A), or in structures resembling multivesicular bodies (MVBs) (fig. 2B, C). In some cases, the nucleocapsids observed in the cytoplasm were surrounded by a thick tegument. Micrographs revealed tegumented nucleocapsids in Golgi cisternae and mainly empty capsids than nucleocapsids in MVBs-like compartments, and tegument-like materials in the cell cytosol, separated from cell membrane structures. The observed MVBs surrounded the cisternae of Golgi apparatus. The virus-like particles noticed within the extracellular space were located near the electron-dense filaments of collagen fibers (fig. 2B).

**FIGURE 1: SEMITHIN SECTION OF THYROID FOLLICLE**



Abundant apoptotic cells and parenchymal destruction, lymphocytic infiltration /arrowheads/, decreased number of follicular cells (black arrows), toluidine blue.

**FIGURE 2: ULTRATHIN SECTIONS OF DURCUPAN-EMBEDDED FOLLICULAR THYROID CELLS**



Viral-like particles (4-point star) in and near infected follicular thyroid cells (a, b), nucleocapsids in the cisternae of several profiles of the golgi apparatus (a). HHV -6 -like particles in the extracellular space near collagen fibrils and mvb-like membrane structures (arrows) in cell cytoplasm with empty capsids (b). Viral-like particles and tegument-like materials in structures resembling multivesicular bodies (MVBs) (top left) scale bar equals: 300nm (a), 600nm (b), 200nm (c).

Potential virions (virus-like particles) found in the extracellular matrix were morphologically characteristic of mature HHV-6 types. The virus-like particles' size was estimated from 100 nm to 160 nm (average size 130 nm) which matched with the HHV-6 capsid and virion size.

## 5. CONCLUSIONS AND DISCUSSION

Studies *in vitro* have reported, that herpes viruses, replicates or have tropism to different cell types - CD4<sup>+</sup>-positive T lymphocytes, CD8<sup>+</sup> T cells,  $\gamma\delta$  T cells, B cells,

natural killer cells, macrophages and megakaryocytes (Ablashi *et al.* 1988; Levy *et al.* 1990; Kondo *et al.* 1991; Lusso *et al.* 1988; 1993 Maric *et al.* 2004). Besides, HHV-6 is considered to be as ubiquitous virus and has possibility to infect almost every cell in the host's organism, including thyrocytes, as it uses CD46 receptor for the cell entry.

Currently, more and more evidence is appearing on HHV-6 involvement in AITD development. It was shown that HHV-6 can persist in thyroid gland and markers of active infection were found (Sultanova *et al.* 2017). Also, similar results were shown by the Italian group from Ferrara University. Furthermore, these researchers have proposed a potential mechanism for HHV-6-induced autoimmunity, by demonstrating that, follicle cells infected with HHV-6 became susceptible to NK-mediated killing (Caselli *et al.* 2012).

Thyroid lesions that were found during the light microscopic observations, were associated with the inflammation process and consisting of focal lymphoid infiltrates, occurred in the presence ELISA-detectable circulating anti-thyroid antibodies, marking autoimmunity. To acquire evidence of active HHV-6 infection it was decided to investigate ultrastructural thyroid gland tissues from AIT patients with molecular markers of active infection. As other herpesviruses, naked nucleocapsids were released into the cytoplasm necessitate tegumentation and re-envelopment with phospholipid bilayer and viral glycoproteins. As it was reported (Maric *et al.* 2004) the tegument layer of the viral particles of HHV-6 (and HHV-7) appears to be very prominent and that characteristic feature distinguishes these types from other herpesviruses using transmission electron microscopy. We were able to observe such similar inclusions - unenveloped, only tegumented and enveloped tegumented nucleocapsids in the cytoplasm and Golgi cisternae of thyrocytes and enveloped tegumented virus-like particles in the extracellular matrix, which is consistent with the data of re-envelopment in the process of viral maturation, membrane fusion and cell egress of herpes viruses. We also note cytoplasmic MVB-like membrane structures, characteristic of herpes infection as reported in an active HHV-6 infection (Mori *et al.* 2008). Our electron microscopic examinations showed cells containing formed MVB-like compartments that included many small vesicles, similar to other studies (Mori *et al.* 2008) and confirmed HHV-6-infection. Our findings are consistent with the suggestion of other authors, that these structures play a role in HHV-6 maturation and take part of cellular exosomal pathway for virion extracellular release.

It is still required to use more specific methods to distinguish HHV-6 particles *in vivo* samples and one of them could be electron microscopy with HHV-6 specific antibodies conjugated with golden particles. This could also resolve another problem with detection of HHV-6 viral particles as this virus is not very productive (in average viral load detected in thyroid tissues' DNA samples was about few thousand copies per million cells). Such small viral loads could indicate on small amount of virions produced during active phase, which makes it difficult to find HHV-6 ultra structures in tissues using electron microscopy.

Overall, detected HHV viral-like particles confirm activity of the virus in thyroid gland tissues, which could indicate the important role of the viral infection in the AITD development. However, to specify HHV-6 viral particles specific immunostaining together with electron microscopy should be carried out.

Hence, we concluded that the presence of HHV virus-like particles in thyroid glands and the morphological findings observed could be related to HHV-6 infection which has higher association and particular significance for the progress of AITD conditions.

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