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Biological Risks to Farmers and Animal Breeders in Lombardy Region, North of Italy

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Biological Risks to Farmers and Animal Breeders in Lombardy Region, North of Italy

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Università degli Studi di Milano a thesis entitled: *Biological Risks to Farmers and Animal Breeders in Lombardy Region, North of Italy*, in fulfillment of the requirements for the degree of Doctor of Philosophy (Occupational Medicine and Industrial Hygiene) of the Università degli Studi di Milano.

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DECLARATION

AND

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I, Ramin Tabibi, declare that this thesis is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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DEDICATION

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ABSTRACT

Objectives: Animal breeding is associated with exposure to a wide variety of risk factors including zoonotic agents, organic dusts, endotoxins, allergens and other chemicals. This PhD project has been addressed at characterizing, at different levels, exposure to biological risks of farmers and animal breeders in the Region of Lombardy. The project has been developed in three main studies, as follows: 1. characterization of biological risks in animal breeders, with a particular focus on selected virus and bacteria; 2. Definition of the anti-tetanus coverage among agricultural workers of the Region in selected population subgroups; 3. study of the immune system function in these workers, through the determination of the serum concentrations of specific cytokines.

Methods: Two groups of farmers and animal breeders were included in the studies. Blood samples were collected from all the subjects and analysed for the presence of the following antibodies against zoonotic agents: *Hepatitis E Virus* (HEV), *Salmonella, Brucella, Coxiella, Leptospira and Borrelia*. In addition, selected serum parameters were measured, including cytokines IL-6, IL-8, IL-10, IFNγ and TNFα, immunoglobulins and proteins, and total and differential white blood cell counts. In addition, level of Immunity to tetanus was investigated. Lung function was measured using a spirometer. Data was analysed using SPSS (version 19) and STATA (version 11) softwares.

Results: Prevalences of antibodies against *HEV, Leptospira spp., Coxiella spp.* and *Borrelia* spp., were 1.0, 54.7, 44.2 and 9.4%, respectively. Animal breeder workers had higher rates of IgG antibodies against *Coxiella* (51.6% *vs* 28.1%, P-value: 0.09) and *Leptospira* (59.4% vs. 43.7%, P-Value: 0.39) than farmers. None of the subjects showed antibodies against *Salmonella spp* and *Brucella spp.* Italian and other European workers had higher immunity to tetanus (93%) in comparison with non-EU workers (77.8%, $P<0.01$). There was a significant increase of TNF-α, IL-8 and IL-10 in animal breeders, and pig breeders had the highest values. Linear regression analysis revealed that there was a statistically difference between EU and Non-EU workers for some spirometry parameters.

Conclusion: Higher titers of antibodies to zoonotic agents in animal breeders indicates that they are probably more exposed to biological agents than other workers who are not involved in animal breeding activities. There must be more attention to the immunity status of tetanus among agricultural workers and in particular migrant workers and occupational health physicians should investigate about the history of immunization during health surveillance activities. The findings of the present study suggest a condition of immune system activation in animal breeders, with the highest levels observed in pig breeders. These changes may be attributable to exposure to organic dusts, endotoxins, or to the different biological agents present in the rural environment. In the curent study we did not point out any significant alteration in the values of spirometry tests between animal breeders and farmers. This data might suggest that, in the conditions of exposure of these workers, only slight sublclinical and adaptive changes are anticipated, but not conditions of overt disease. Further studies with larger sample size are needed to better understand the mechanisms for transmission of zoonotic infections and their potential reservoirs in northern Italy.

Keywords: *Zoonoses, Zoonotic agents, agricultural workers, animal breeders, Serum cytokines, Heptatits E virus, Tetanus immunity, Lombardy, Italy.*

RIASSUNTO

Obiettivi: l'allevamento degli animali è associata all'esposizione ad una vasta gamma di fattori di rischio tra cui agenti zoonotici, polveri organiche, endotossine, allergeni e altre sostanze chimiche. Il presente progetto di dottorato di ricerca intende descrive, nei suoi diversi aspetti, l'esposizione a rischio biologico degli agricoltori e allevatori in regione Lombardia. Il progetto è stato sviluppato in tre studi principali, così come indicato di seguito: 1. Descrizione del rischio biologico negli allevatori, con un'attenzione particolare a virus e batteri selezionati, 2. Definizione della copertura antitetanica tra i lavoratori agricoli lombardi in sottogruppi di popolazione selezionati; 3. Studio della funzione del sistema immunitario in questi lavoratori, attraverso la determinazione delle concentrazioni sieriche di specifiche citochine.

Metodi: Sono stati coinvolti negli studi due gruppi di agricoltori e allevatori. Per ciascun soggetto è stato raccolto un campione di sangue, e ciascun campione è stato poi analizzato per verificare la presenza dei seguenti anticorpi contro i seguenti agenti zoonotici: virus dell'epatite E (*HEV*), *Salmonella*, *Brucella*, *Coxiella*, *Borrelia e Leptospira*. Inoltre, sono stati misurati alcuni parametri sierici selezionati, comprese citochine IL-6, IL-8, IL-10, IFNγ, TNF-α, immunoglobuline e proteine, e conta totale e differenziale dei globuli bianchi. E' stato studiato il livello di Immunità al tetano. La Funzione polmonare è stata misurata con uno spirometro. I dati sono stati analizzati utilizzando SPSS (versione 19) e Stata (versione 11) software.

Risultati: Si è registrata una prevalenza di anticorpi anti *HEV, Leptospira spp, Coxiella spp. e Borrelia* spp., rispettivamente nelle percentuali del:1,0, 54,7, 44,2 e 9,4%. Rispetto agli agricoltori gli allevatori avevano tassi più elevati di anticorpi IgG contro *Coxiella* (51,6% vs 28,1%, P-value: 0,09) e *Leptospira* (59,4%, 43,7%, P-value: 0,39). Nessuno dei soggetti ha mostrato anticorpi contro *Salmonella* spp e *Brucella*. I lavoratori, italiani ed europei avevano un più alto stato di immunizzazione contro il tetano (93%) rispetto ai lavoratori non comunitari (77,8%, p <0.01). Vi è un significativo aumento di TNF- α, IL-8 e IL-10 negli allevatori, con i valori più alti tra gli allevatori di suini. L'analisi di regressione lineare ha rivelato che c'era una differenza statistica tra i lavoratori dell'UE e i lavoratori extracomunitari per alcuni parametri spirometrici.

Conclusioni: alti titoli di anticorpi contro gli agenti zoonotici negli allevatori indicano che sono probabilmente più esposti ad agenti biologici rispetto agli altri lavoratori che non sono coinvolti in attività di allevamento di animali. Ci deve essere una maggiore attenzione allo stato immunitario del tetano tra i lavoratori agricoli, in particolare i lavoratori migranti; i Medici del Lavoro dovrebbero indagare sulla storia di immunizzazione durante le attività di sorveglianza sanitaria. I risultati dello studio presentato suggeriscono una condizione di attivazione del sistema immunitario negli allevatori, con i più alti livelli osservati tra gli allevatori di suini. Queste lievi alterazioni possono essere attribuibili all'esposizione a polveri organiche, endotossine, o ai diversi agenti biologici presenti nell'ambiente rurale. Il presente studio non ha evidenziato differenze significative nei valori dei test spirometrici tra gli allevatori e gli agricoltori. Questo dato potrebbe suggerire che, nelle condizioni di esposizione considerate, si manifestino modificazioni di tipo adattivo ma non patologie conclamate. Ulteriori studi che prendano in considerazione campione di dimensioni maggiori sono necessari per comprendere meglio i meccanismi per la trasmissione di infezioni zoonotiche e dei loro potenziali bacini del Nord Italia.

Parole chiave: *Zoonosi, agenti zoonotici, lavoratori agricoli, allevatori, citochine sieriche, Hepatitis virus E, immunità tetano, Lombardia, Italia.*

List of Articles

My thesis is mainly based on five published articles (one still under revision) as outlined below. There are also some additional unpublished materials which are included in the thesis.

1. Tabibi R, Consonni D, Brambilla G, Melzi d'Eril G, Sokooti M, Romanò L, Somaruga C, Vellere F, Varischi G, Colosi. Rischio di zoonosi per i lavoratori agricoli Lombardi. *G Ital Med Lav Ergon* 2012; 34:3(2 Suppl):164–165.

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3. Giulia Rabozzi, Luigi Bonizzi, Eleonora Crespi, Chiara Somaruga, Maryam Sokooti,Ramin Tabibi, Francesca Vellere, Gabri Brambilla, Claudio Colosio. Emerging Zoonoses: the "One Health Approach". *Saf Health Work*; 2012;3(1):77–83.

4. R. Tabibi, E. Corsini, C. Somaruga, M. Sokooti, G. Rabozzi, F. Vellere, C. Colosio,G. Brambilla. Occupational exposure to biohazards and endotoxins among agricultural workers in the region of Lombardy, Northern Italy. *Toxicology Letters* 2011;205,. p. S151.

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6. R. Tabibi, R. Baccalini, A. Barassi, L, Bonizzi, G. Brambilla, D. Consonni, G. Melzi d’Eril, L. Romanò, M. Sokooti, C. Somaruga, F. Vellere , A. Zanetti, C. Colosio. Occupational Exposure to Zoonotic Agents Among Agricultural Workers in Lombardy Region, Northern Italy. (Submitted to *Annals of agriculture and Environmental medicine,* 2012).

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ACRONYMS AND ABBREVIATIONS

Ab ELISA	Antibody detection Enzyme Linked Immunosorbent Assay
ASCC	Australian Safety and Compensation Council
BAL	Bronchoalveloar Lavage
BSE	Bovine Spongiform Encephalopathy
CCHF	Crimean–Congo hemorrhagic fever
CDC	Centre for Disease Control and Prevention
CI	Confidence Interval
COPD	Chronic obstructive pulmonary disease
ELISA	Enzyme Linked Immunosorbent Assay
FEF 25-75%	Forced expiratory flow
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
HEV	Hepatitis E virus
HPA	Health Protection Agency (UK)
ILO	International Labour Organization
ISTAT	Italian Institute for Statistics
LFT	Lung function test
MEF25%	Maximal expiratory flow
MSD	Musculoskeletal disorders
ODTS	Organic Dust Toxic Syndrome
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
PEF	Peak expiratory flow
PHC	Primary Health Care
PIF	Peak Inspiratory Flow.
PPE	Personnel Protective Equipment
SD	Standard Deviation
TB	Tuberculosis
TSE	Transmissible Spongiform
UAA	Utilised Agricultural Area
vCJD	Variant Creutzfeldt-Jakob Disease
WHO	World Health Organisation
WNV	West Nile Virus

Chapter I Introduction

1.0 General Introduction

1.1 Agriculture

The agricultural sector employs an estimated 1.3 billion workers worldwide, which is half of the world's labour force. In terms of fatalities, injuries and work-related ill-health, it is one of the three most hazardous sectors of activity worldwide (along with construction and mining). According to ILO estimates, at least 170,000 agricultural workers are killed by occupational accidents each year. Farming is one of the few industries in which the families (who often share the work and live on the premises) are also at risk for fatal and nonfatal injuries. It is also the largest sector for female employment in many countries, especially in Africa and Asia (ILO website).

Much agricultural work is, by its nature, physically demanding. The risk of accidents is increased by fatigue, exposure to extreme weather conditions, poorly designed tools, difficult terrain and poor general health, associated with working and living in remote and rural communities. Agricultural work is accompanied by co-occurrence of many risk factors threatening farmers' health, e.g. dust, elements of the thermal environment, noise, vibration, chemical and biological agents. Biological agents can cause diseases of infectious allergic or immunotoxic background which constitute the majority of farmers' occupational diseases. Exposure to these factors in the agricultural working environment is due to contact with animals, organic wastes, plants and more precisely with microorganisms, plant and animal particles present in aerogenic agricultural dust, as well as pathogens of contagious and invasive diseases present in soil, water and plants (Mołocznik 2004, ILO website).

Biological hazards are different kind of organic substances that pose a threat to the health of humans and other living species. In agriculture there are various sources of biological hazards and materials that the farmers and workers can be exposed to. The specific biological agents in agricultural work include bacteria, fungi, mites and viruses transmitted from the animals, parasites and ticks. Certain species of fungi produce very toxic toxins, which can be found in agricultural materials and in the air of animal houses. Animal breeding facilities are usually contaminated by organic dusts, characterized by diverse composition but usually containing biological agents and some of their products, including endotoxins, which, produced by certain bacteria, bring about a significant risk to respiratory organs. Other typical components of organic dusts are part of vegetables and animals, pollens, mitogen substances, etc. Natural or organic materials such as plants and soil may pose risks to workers. In grain dust, which can cause respiratory disease, the main component is the grain itself. Materials harvested for animal feed may contain moulds, actinobacteria, and bacteria, especially when stored wet. Materials of animal origin such as hair, dander, skin debris from cows, pigs, poultry and dust particles from flour also give rise to a risk of occupational disease.

Workers may be exposed to infected animals and be infected by zoonotic diseases. Storage mites are very allergenic for humans and the agricultural environment is suitable for their growth. In general, workers at risk include e.g. animal handlers, grain handlers, root crop workers, mushroom workers and veterinarians. Worldwide, it is estimated that around 320,000 workers die each year from communicable diseases caused by work-related exposures to biological hazards (European Agency for safety and Health at Work, 2012; Driscoll *et al*. 2005; OSHA 2007).

1.1.2 The Agriculture in Italy

There are 1,620,844 agricultural and zootechnical farms active in Italy (-32.4 % compared to 2000). The medium size of farms is around 7.9 hectares of utilized agricultural area (UAA) (+44.2%). The total UAA is equal to 42.8% of the national territory (12.9 million of total hectares), with a reduction of 2.5% compared to 2000. The Italian agricultural and zootechnical structure, even though continues to be based on single or family enterprise (96.1%) where the direct conduction of the enterprise are the predominant form (95.4%). On a total of 217,449 breeding farms, 124 thousand do bovine breeding with a share of 57.1% of the zootechnical sector. This type of breeding is mostly seen in the Northern part of the country, in particular in Lombardy, Piedmont, Veneto and Emilia Romagna. On the whole these four regions possess a little less than two third (64.6%) of the Italian bovine assets. According to the 6th general census on agriculture, the number of agricultural farms decreases, but there is an increase in their average size. Around 99% of the agricultural farms are managed by families and 30.7% by women. Confirming the traditional structure of the Italian agriculture, the results of the 6th census shows a significant signs of change highlighting a sector in slow but clear social-economic evolution. In ten years the labour force has decreased by 50.9 %, with a significant increase of the proportion of aged workers (whose quota passes from 14.3% to 24.2% in 2000-2010). The quota of female labour is 37%.

The 2010 census for the first time had information on the foreigners working in agriculture, their presence results to be increasing. In particular, the foreign workers are 233 thousand people and they are 24.8% of the farm labour force in non family run farms and 6.4% in total. 57.7% of the foreign labour force comes from EU countries while 42.3% from non EU countries. 30.7% of the agricultural farms are managed by women. The number of farms managed by foreigners is very small (0.1%) with higher values in the South (0.6%). There are 44455 organic farms. 63% of these are located in the South of Italy (Agricoltura Italiana website).

The following figure (1-1) shows a comparison between the utilised agricultural areas by size (ha) in Italy in the years of 2005 and 2007.

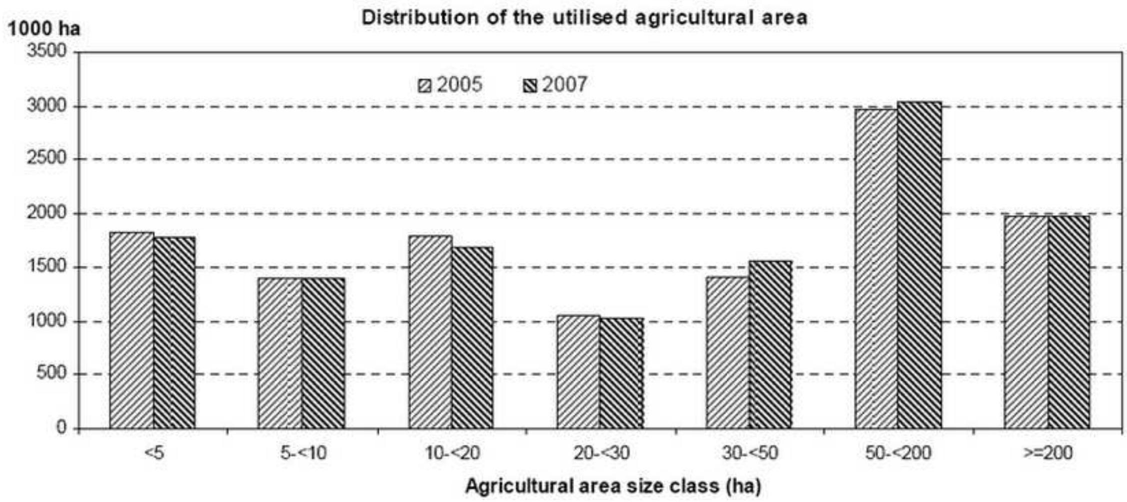

Fig1-1. Distribution of the utilised agricultural area, Italy in 2005 and 2007 (Data from EUROSTAT)

1.1.3 The Lombardy Region

The Lombardy Region is considered as one of the highest economic growth rates in Europe, expressed in Gross Domestic Product (GDP) per capita (EU, 2010). At the same time, this region is characterized by a mounting process of urbanization (EEA, 2006) which is causing the fragmentation of forest areas (Carovigno *et al.*, 2011). The total surface of the Region of Lombardy is 2.386 million hectares of which more than the 70% are seed fields. In Lombardy, the average UAA is about 14 ha per enterprise, about 41,000 companies have less than 5 ha of UAA, with a coverage ratio of 6.5% of the surface, while enterprises with more than 20 ha of UAA are 12,349 and cover about 75% of UAA itself.

The trend towards the enterprises' growth in the size is demonstrated by the fact that compared to 1990 enterprises with more than 50 ha of UAA cover 50.5% of the surface against the previous 40.3%. The Farm structure survey recorded 1,680,000 agricultural holdings in Italy in 2007 (3% less than in 2005). The labour force involved in agriculture activity has fallen by 5%, while the utilized agricultural area (UAA) and the total of livestock have increased by 0.3 % and 4%, respectively (ISTAT website). Farms and agricultural areas in Lombardy Region are shown in **Table 1-1**.

Table 1-1. Farms and agricultural area in Lombardy Region (ha). (Source: ISTAT)

Province	Farms(No.)	Area (ha)	Average surface
Varese	1663	20,613.65	12.40
Como	2008	34,062.39	16.96
Sondrio	7450	186,605.87	25.05
Milan	4679	91,689.63	19.60
Bergamo	10,349	140,695.97	13.60
Brescia	17,167	308,267.22	17.96
Pavia	11,222	220,155.24	19.62
Cremona	5117	142,955.10	27.94
Mantua	11,819	190,393.51	16.11
Lecco	1241	14,902.22	12.01
Lodi	1786	63,073.97	35.32
Lombardy	**74,501**	**1,413,415**	**18.97**

1.2.0 The Livestock

The livestock sector has experienced an amazing growth in the last decade fuelled mainly by global expansion in demand for food of animal origin. This has been attributed primarily to population growth, urbanisation and income growth and has since been coined the 'livestock revolution' (Delgado *et al* 1999). In 1995, for the first time, meat volume produced in the developing countries exceeded that of developed countries and since then the gap in milk output between developing and developed countries has been narrowing (FAO report, 2006). The livestock revolution has implications for our health, livelihoods, and environment. Livestock produce food, provide security, enhance crop production, produce cash incomes for rural and urban populations and generate value added goods which can have multiplier effects and create a need for services. Livestock also form a major capital reserve of farming households. Because of livestock's donation to societies, human and economic pressures can direct livestock production in detrimental ways to the environment (Chilonda *et al.*, 2006; FAO, 1996).

In majority of developing countries, livestock keeping is a multifunctional activity. In addition to their direct role in generating food and income, livestock are a valuable asset, serving as a store of wealth and a crucial safety net during times of crisis. Animal source foods are mainly rich sources of necessary nutrients, and relatively small amounts of these foods, added to a vegetarian diet, can significantly increase nutrient adequacy (Murphy and Allen, 2003). Globally, pig and poultry production are the quickest growing and industrializing livestock sub-sectors with annual production growth rates of 2.6 and 3.7 percent over the past decade. In industrialized countries, the vast majority of chickens and turkeys are now produced in houses in which between 15,000 and 50,000 birds are kept throughout their lifespan. Increasingly, quail, pigs, and cattle are also raised under similar conditions of high density. This trend towards industrialization of livestock production is also occurring in developing countries, where intensive production is rapidly replacing traditional systems, principally in Asia, South America, and North Africa (Ranald and Cameron, 2000).

1.2.1 The Livestock in Italy

The livestock sector plays a crucial role in the EU in general and particularly in Italy, both from a purely trade and production aspect. The livestock industry is accounted as the base of the primary production of food of animal origin, for the humans and is affected not only by economic and trade problems, but also by ecological and health problems. Data on farms show remarkable differences between Regions, both for the number of enterprises engaged in this activity and for the number of animals bred.

In 2008, in Italy there were 7,211,000 head of cattle, 8,329,000 hogs, 11,089,000 sheep, 1,375,000 goats, 285,000 horses, and an estimated 100 million chickens. That year, total meat production from hogs, cattle, sheep, and goats was 4,141,000 tons. Of the meat produced, 36% was pork, 27% was beef, 27% was poultry, 1% was mutton, and 9% was from other sources. Meat production is lower than domestic requirements, and about half of all meat consumed must be imported. Although Italy produced 11.3 million tons of cow milk in 2008, dairy farming remains comparatively undeveloped. Both dairy and beef cattle are raised mainly in the North part of the country.

The ISTAT survey on the structure and production of farms shows that in North Italy pig breeding represents the majority of breeding facilities (85% of the national total), with three quarters of this national consistency species bred in only three regions: Lombardy (48.2%), Emilia Romagna (15.6%) and Piedmont (11.0%). The sheep and goats breeding however, traditionally are bred in the Southern regions (respectively 72.4% and 75.4% of the national total), and in particular in the region of Sardinia. It has to be mentioned that 81.3% of the poultry sector is in the North with particularly 28.6% in the region of Veneto, of 23.8% in Lombardy and the rest in Emilia-Romagna.

1.2.2 The Livestock in the Region of Lombardy

The vocation regional livestock is well expressed by the number of farms with livestock and large herds of bovines, with 1,618,000 animals and swine with 3700,000 heads and also poultry livestock with more than 90,000,000 of animals. Among the productions, the most representative is the milk production with 39 million tons that is more than the 36% of the national milk production.

With regard to pig sector Lombardy accounts for almost 50% of national production; with Emilia-Romagna (16%) and Piedmont (11%), these 3 regions account for more than ¾ of pigs reared in Italy. A very strong concentration of pig farms is located in the Provinces of Mantova and Brescia With regard to poultry, Veneto accounts for 29%, Lombardy 24% and Emilia-Romagna 19% of the national total poultry number in Italy. In the 5 regions of Northern Italy 76 million broilers and 29 million laying hens are reared. 10% of farms account for 77% of broilers; 3% of laying hen farms (exceeding 25000 heads) accounts for 90% of the total number of poultry heads.

Cattle and pig production of Northern Italian Regions, compared to European Countries with similar level of intensity, is characterised by absence of grazing (cattle sector) and by predominance of fattening cycle up to a slaughtering weight of 150-160 kg (pig sector). Pig fattening was traditionally linked to cheese production, allowing utilisation of whey. For pigs, Lombardy reaches the maximum number of farms and hectares, followed by Piedmont, Emilia-Romagna and Friuli Venezia Giulia have the lowest percentages but livestock refers to very specialised agricultural areas: Parmigiano-Reggiano cheese, Parma ham, San Daniele ham. As one region addressed prevailing to the livestock, Lombardy sees its surface mainly cultivated at cereals, maize and barley, which are the main source of feed for the livestock. (Ministry for Environment Land and Sea, 2010).

Table 1-2 compares the number of farms and cattle in Lombardy Region and other parts of the Country.

Table 1-2. Cattle farms in Italy and the Region of Lombardy (ISTAT, 2010).

	Cattle farms		Number of animals	
	2010	2000	2010	2000
Lombardy	14.700	19.684	1.483.557	1.606.285
Italy	124.341	171.994	5.677.953	6.049.252
North-West	30.198	41.509	2.346.246	2.480.904
North-East	32.259	48.736	1.652.307	1.849.410
Center	18.007	24.476	429.394	477.572
South	26.892	39.543	662.616	684.140
Islands	16.985	17.730	587.390	557.226

1.3.0 Health Risks to Agricultural workers

In addition to safety hazards on a farm, such as tractors, harvesters or balers, there are also health hazards that can cause a work-related disease. The main workplace health hazards are chemical, physical, biological agents. Exposure to such agents can have serious and immediate consequences as well as chronic or long-term consequences.

1.3.1 Chemical Agents

They are considered not only pesticides and other agrochemicals used, but also fuels, solvents, detergents, disinfectants and antibiotics (found in medicated feed administered to farmed animals). Good management, use, and disposal of agrochemicals, especially pesticides, is an important health and environment issue in developing countries, where economies may be heavily based on agriculture. The issue is a focus of this section on toxic hazards. Pesticides in general (plant protection products, agrochemicals), are chemical compounds specifically manufactured to be toxic to target organisms, deliberately introduced into the environment in large amounts and used by several million workers all over the world.

The main chemical classes of pesticides are the following:

- Organophosphorous compounds (OP, insecticides and herbicides)
- Carbamates (insecticides)
- Pyrethroids (insecticides)
- Dithiocarbamates (fungicides)
- Organochlorinated compounds (different uses)
- Quaternary ammonium compounds (herbicides)
- Phenoxy acids derivatives (herbicides)
- Coumarine derivatives (rondenticides)
- Conazoles (fungicides)

(ILO Encyclopaedia, Brawn *et al.*, 2009, European comission 2009)

Acute exposure to pesticides can lead to death or serious illness (WHO website). Chronic pesticide exposure is mainly a problem in the occupational setting, principally among poor rural populations where men, women and children all work and live in close proximity to fields and orchards where chemicals are applied and stored (Goldman and Tran, 2002; FAO/UNEP/WHO, 2004). Long-term exposure to pesticides can increase the risk of developmental and reproductive disorders, endocrine disruption, immune-system disruption, impaired nervous-system function, and development of certain cancers. Children comparing to adults are at higher risk from exposure (WHO website; Yáñez, 2002). Pesticides, when not properly used, may disrupt natural biological pest control mechanisms. More vigorous pest attacks may result, along with heavier chemical use, and increased health exposures. Pesticides, like fertilizers, can infiltrate water sources contaminating drinking water and animal species, e.g. fish, upon which humans rely for nutrition. This contamination can lead to a range of secondary public health impacts (FAO/UNEP/WHO, 2004; UNEP 1999).

In developing countries, deaths by unintentional poisoning may be strongly linked with inappropriate use and poor environmental management of toxic chemicals, including pesticides. The agricultural worker is exposed to pesticides during different stages of the work-cycle, from the application of the pesticide on vegetable products and fruit to the pick fruit with a potential increase of the risk of exposure often linked to the absent or inefficient formation and information of workers (especially seasonal workers) (Giorgi, *et al.,* 2012).

Pesticides have contributed to impressive increases in crop yields and in the quantity and variety of the diet. Also, they have helped to limit the spread of specific diseases. But pesticides have harmful effects; they may cause injury to human health and also to the environment. The range of these adverse health effects includes acute and persistent injury to the nervous system, lung damage, dysfunction of the immune and endocrine systems, injury to the reproductive organs, birth defects, and cancer. Problems related to pesticide hazards to man and the environment are not confined to the developing countries. Developed nations have already suffered these problems, and still facing some problems in certain locations (Mansour, 2004).

1.3.2 Physical Agents

Physical hazards to agricultural workers may include extreme heat, extreme cold, lightning noise, vibration and ultraviolet radiation (UV). Extreme heat situation can cause heat stroke, heat cramps, heat exhaustion, heat rash and other problems. Extreme cold conditions may cause, frostbite, hypothermia and other problems. Too much noise exposure may cause a temporary change in hearing or a temporary ringing in workers' ears (tinnitus). Frequent exposures to loud noise can result in permanent, incurable hearing loss or tinnitus. Lightning kills about 80 people in the United States each year and injures hundreds. Among construction workers, laborers, engineers, machine operators, roofers, and pipefitters have been struck by lightning most often on the job. UV radiation may cause problems such as sunburn and skin cancer (CDC website). The other risk factor of this group is the musculoskeletal system such as the manual handling of loads and the repetitive movements. Musculoskeletal disorders (MSDs) are increasingly accepted as a significant hazard of agricultural occupation. In agricultural jobs with significant physical labor, MSDs are typically the most frequently reported injury and result in disability, lost work time, and increased production costs. MSDs increase production costs as a result of worker absence, insurance and medical costs, decreased work capacity, and loss of employees to turnover and competition from other less physically demanding industries (Kirkhorn *et al.*, 2010).

Figure 1-2 illustrates the trend of incidence rates for occupational accidents for the two sectors of industry- services and Agriculture during 1951-2001 in Italy (Baldasseroni *et al*., 2005).

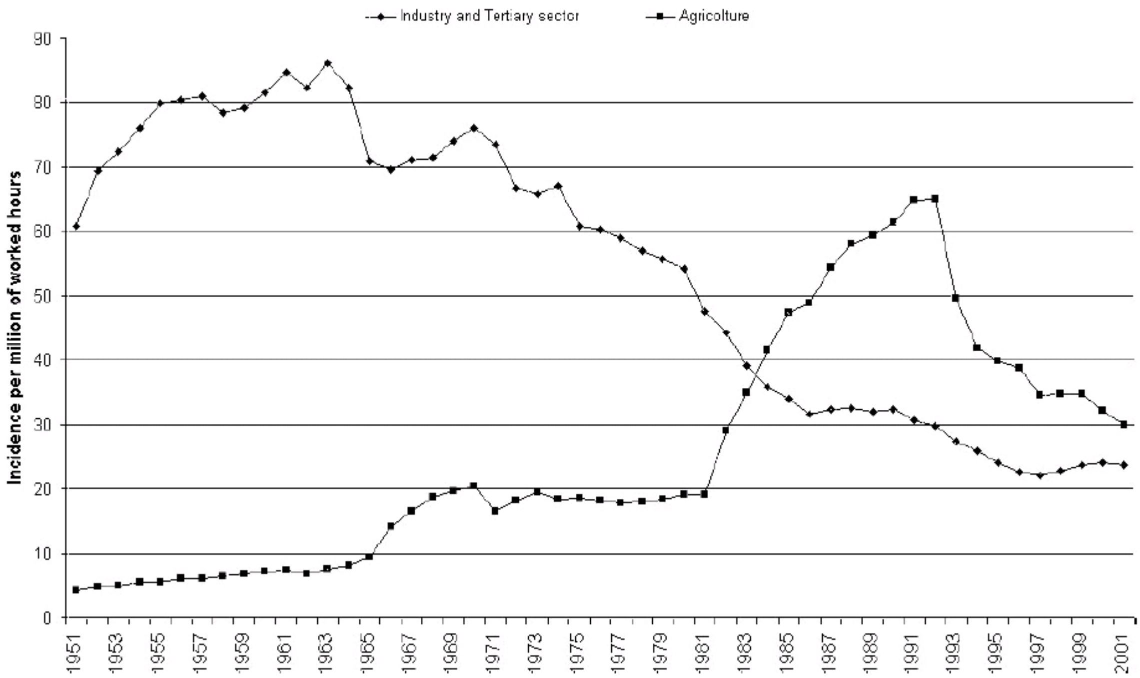

Fig. 1-2. Yearly trends of incidence rates for occupational accidents, 1951–2001, by sector (Source: Baldasseroni *et al.*, 2005*).*

1.3.3 Biological Agents

Biological hazards can be important sources of ill health in agriculture. In agriculture, forestry and animal food production, workers are mainly at risk from:

- Bacteria, fungi, mites, and viruses transmitted from animals, parasites and ticks (zoonoses)
- Respiratory problems due to microorganisms and mites in organic dusts of grain, milk powder, flour, spices and specific allergic diseases like farmer's lung and bird breeder's lung (OSHA website).

Agricultural workers may come into contact with these biological agents through routine exposures, such as from contact with animals, contaminated water, animal carcasses, or working in or near livestock houses and stabling areas. The fact that people working with animals or their products may contract some infections has been known for centuries, before the introduction of the concept of zoonoses. Only recently, the prevention of these occupational risks has been taken into account by legislation in spite of the fact that some zoonoses of livestock have significant socio-economic impact.

Nowadays some factors such as new production technologies, trade globalisation, movements of people, changes in working conditions, are generating new zoonotic and occupational risks, some of which are considered re-emerging. The prevention of occupational zoonoses must be implemented jointly by both veterinary and medical services through prevention and epidemiological surveillance of human and animal health, risk evaluation, diagnosis of infections and safety at work (Battelli *et al*., 2006).

In 1975, a joint WHO/FAO meeting of VPH (veterinary public health) experts recognized zoonoses as occupational risks and stressed the need for specific knowledge for securing their prevention and control. At the meeting, the committee, upon Italian proposal, classified zoonoses from the socio-economic viewpoint as follows: 1) zoonoses with serious effects on animal production; 2) zoonoses with serious consequences both for man and for economically important animals; 3) zoonoses with serious consequences for man, but much less serious in economically important animals. This type of classification takes into account the socio-sanitary and socio-economic significance of zoonoses. Many zoonoses possibly associated with occupational activities (brucellosis, bovine tuberculosis, anthrax, dermatomycosis, leptospirosis) belong to the first two categories (Bellani *et al*., 1978). Several occupational diseases possibly involving personnel working in animal husbandry and related activities are described (Mantovani *et al*., 1978; Battelli *et al*., 1984)

1.3.3.1 Zoonoses

Evidence suggests that 75 percent of emerging pathogens and 61 percent of all infectious organisms are zoonotic (Taylor *et al.,* 2001). Important recent examples include the emergence of novel influenza virus H1N1 in the United States and Mexico in 2009, with rapid progression into a universal pandemic, the introduction of monkeypox into the United States from Africa in 2003 and the severe acute respiratory syndrome (SARS) epidemic in 2002–2003 (Miller and Heptonstall, 2010; Peters *et al.,* 2008). Therefore, biological agents of animal origin may lead to an occupational health risk, involving subjects who are in contact with animals or animal derivatives such as breeders, butchers, abattoir workers and veterinarians. According to Cozzi and Ragno (2003) biological agents accompany nearly 60% of farm activities and farmers generally are not aware of their presence.

The World Health Organisation (WHO) defines zoonoses as infections and diseases that are naturally transmitted between vertebrate animals and humans. A zoonotic agent may be a virus, bacterium, a fungus or other communicable disease agent. Apart from the newly emerging zoonoses such as highly pathogenic avian influenza (H5N1) and Severe Acute Respiratory Syndrome (SARS) the vast majority are not accorded a high level of priority by national and international healthcare systems. According to Stephen *et al* (2004), zoonoses are fundamental determinants of community health so preventing, identifying and control of these infections must be a central public health focus.

Many factors lead to the emergence of zoonotic diseases; Environmental changes, human and animal demography, pathogen changes and changes in farming practice are a few of them. Social and cultural factors such as food habits and religious beliefs also play a role. (WHO website). According to Brun *et al* (2007) **“**when a pathogen emerges, given the speed and volume of international traffic and trade, it may spread around the world within a few hours and start a new pandemic" .Many zoonotic diseases impact significantly on both human health and livestock productivity.

The livestock industry is particularly important to the economy of every country and includes not only commercial producers of milk or meat, but also purebred breeders and small producers with a few animals. The success of livestock operation is closely related to the healthiness of the animals. Losses due to disease in livestock originate in many ways. Some are obvious, such as death of animals, medication costs, and removal of diseased animals at the processing plants. Others might be less obvious, such as poor production, poor growth, poor feed, conversion, and downgrading of quality of products. Biological risk management is the overall process of awareness education regarding the risk of infectious diseases entering or spreading through an animal facility. It also involves evaluating and managing those risks (Hersom *et al.*, 2008).

The lack of information on biological risks in the workplace, which makes risk assessment difficult, has been treated as an emerging risk especially in the agriculture sector and office workplace (Brun, 2007). The control of arthropods and arthropod-borne diseases is also of great importance due to their considerable influence on the productivity of the livestock and the health status of domestic animals. The rising awareness of arthropods as vectors of zoonotic diseases has created a public demand for effective control agents which can also be used safely for the treatment of companion animals and to destroy pests in the direct environment of human beings (Londershausen, 1996).

Vaarst *et al* (2008) investigated animal diseases in organic livestock in Europe and reached the conclusion that the main disease issues seemed to be similar between the European countries, for example metabolic problems, lameness and mastitis in dairy cows. For beef cattle, sheep and pigs, parasitism was mentioned as a main issue, and feather pecking and cannibalism were highlighted as a main challenge in poultry. Many disease problems seemed to be of a smaller scale in South Europe than in North- Western European countries. Based on an interview study of veterinarians with experience in organic livestock farming, it was suggested that the warm, dry climate might explain this.

According to Gregory *et al* (2008) US veterinarians who work with poultry, poultry workers and US hunters are at increased risk of recreational or occupational avian influenza virus infections and also subclinical avian influenza virus infections may be more common than expected (Myers, 2007). Hayden and Croisier (2005) considered similar findings among Italian poultry workers and concluded that the low prevalence of antibody and temporal association with avian influenza epizootics argued for human infection with avian viruses as an explanation.

Existing data suggest that the risk of occupational zoonoses has been underestimated (Rabozzi *et al*., 2012; Battelli, 2008). It is therefore clear that in animal breeding settings, animals', workers' and consumers' health are strongly linked and interdependent. In fact, biological risks are widely represented in agriculture and animal breedings, due to environmental characteristics and injury typology and among infectious diseases that can affect agricultural workers.

Main outbreaks of zoonoses in the past 25 years

In the last decades, some serious animal and human diseases have emerged or re-emerged worldwide. **Table 1-3** indicates the most important epidemics by emerging zoonoses from 1976 till present.

Table 1-3. New and Emerging Zoonotic Diseases from 1976 -2011

Year	Disease Name	Details
1976	Cryptosporidium	Originally thought to be a disease of reptiles and birds. Transmission occurs direct via exposure to infected faeces.
1976	Ebola	Virus found in Central Africa that is transmitted via direct contact with infected bodily fluids. Source of the Ebola virus is currently unknown.
1981	HIV/ AIDS	Since 1981 AIDS has killed more than 25 million people. Currently, 1.1 million Americans are among the 33 million people living with HIV, the virus that causes AIDS.
1982	E. Coli 0157:H7	First recognized as a cause of illness during an outbreak that was traced to hamburgers contaminated with *E. coli* 0157:H7 bacteria.
1993	Sin Nombre Virus	First recognized in 1993 after the investigation of an outbreak of sudden fatal respiratory illness in the southwestern United States.
1999	West Nile Virus	WNV arrives in New York. WNV is now found in 47 States.
2003	SARS	According to WHO, a total of 8,098 people worldwide became sick with SARS during the 2003 outbreak. Of these, 774 died.
2003	Monkey Pox	Monkeypox was reported among several people in the United States.
2003	H5N1 Influenza	Human cases first reported in 2003. Sixty percent of those people reported infected with the virus have died.
2003	Mad Cow Disease	The first case of mad cow disease in the United States discovered.
2009	Novel H1N1 Influenza	In June 2009, the WHO declared the new strain of swine-origin H1N1 as a pandemic.

(Ref Colorado report, 2011)

A global view on emerging zoonoses from 1996–2004 is depicted in **Figure 1-3.**

Fig 1-3. The reported global emerging zoonoses between the years of 1996-2004 (WHO 2007).

Italian Animal Breeding Enterprises and Biological Hazards (zoonoses)

The Italian meat production has a long history and tradition and its weight within the national agriculture gross domestic product (GDP) is around the 25%. About 70% of the Italian pig production is located in the Po Valley and the sole Lombardy region counts for about one third of the entire national swine population (Cozzi and Ragno, 2003).

Pork is the cheapest and most frequently consumed meat in the European Union (Devine, 2003) and the European pig herd is the second largest in the world after the Chinese herd . Pork meat production and consumption is a source of several food-borne diseases; also pig breeders are subject to a wide variety of hazardous exposures, which include physical, chemical and biological agents. In this light, the management of biological risk to workers engaged in the production and pork meat consumers is of major health and economic significance. Of course, due to different exposure modalities, the main biological risk factors to be considered for workers and consumers are in some cases different.

The nutritional quality of pig meal is strictly related to the farm sanitary management, mostly regard to the control of opportunistic bacteriosis (Beloeil, 2004). Besides, these infections are among the major causes of food borne diseases of swine origin in man. Among the most important factors influencing the onset of illness in pig farm, there are animal relocations and changes of production phase (weaning, growing and fattening), the mixing of pigs from different farms, excessive use of antibiotics and the lack of environmental management. Pig-rearing facilities also are one of the most intense sources of environmental microbiological contamination and off-odour emission. Although both micro-organisms and off-odours of agricultural origin are not per se a source of occupational or community health concern, they are nevertheless contentious towards neighbouring populations and a cause of value loss for real estate (Bonetti *et al*., 2002). Moreover, a high level of olfactory nuisance from intensive animal rearing may underpin poor zoo-technical practices. To date very little systematic data exists on environmental microbiological contamination and olfactory emissions of high-impact economic activities in the agricultural sector (Colombi *et al*., 2005).

Zoonoses associated with Animal breeding

As a food animal species, pigs are raised globally but under a variety of production systems. Distribution and occurrence of particular zoonosis in swine is directly linked with this environment (Poljak, 2009). In Europe, twenty-seven biological hazards may be transmitted from pork to consumers (Fosse *et al*., 2008). Pigs can be infected with many biological agents and these agents can cause disease in animals or be in asymptomatic forms. These zoonotic agents can be transmitted from animals to workers of the farms, slaughterhouses, meat processing and waste treatment.

Some common zoonoses which workers can be exposed to are: Influenza, *Brucella suis, Erysipelothrix rhusiopathiae, Leptospirosis spp., Mycobacterium bovis, Yersinia enterocolitica, Cryptosporidium parvum, Giardia intestinalis, Balantidium coli, Streptococus suis, Clostridium tetani , Methicillin Resistant Staphylococcus Aureus (MRSA), Dermatophytosis, Microsporum nanum, Microsporum canis, Trichophyton, mentagrophytes* and *T. Verrucosum* and *Hepatitis E* virus .

HEV

Hepatitis E virus (HEV) is an emerging enteric pathogen responsible for most acute hepatitis worldwide .Hepatitis E virus is a single –stranded, nonenveloped virus with an RNA genome of 7.5 kb (Purcell and Emerson, 2008) and infection caused by hepatitis E virus (HEV), is a common cause of acute hepatitis in areas with poor sanitation. The virus has four genotypes with one serotype: genotypes 1 and 2 exclusively infect humans, whereas genotypes 3 and 4 also infect other animals, particularly pigs. In endemic areas, both large outbreaks of acute hepatitis as well as sporadic cases occur frequently.

These cases are usually due to genotype 1 or 2 HEV and are principally caused by fecal–oral transmission, usually through contamination of drinking water; contaminated food, materno-fetal (vertical spread) and parenteral routes are less common modes of infection. The acute hepatitis caused by this virus has the highest attack rates in young adults and the disease is particularly severe among pregnant women. HEV superinfection can occur among persons with pre-existing chronic liver disease. In non-endemic regions, locally acquired disease was believed to be extremely rare and it is documented that antibody to hepatitis E virus (anti-HEV) is prevalent in western countries, where clinical hepatitis E is rarely reported (Christensen *et al*., 2008). HEV has been the cause of waterborne outbreaks of hepatitis in Asia and Africa and is a major cause of sporadic hepatitis in these regions. Acute infection primarily affects young adults and is generally mild, except in women during late pregnancy, among whom 20% mortality has been reported.

However, in recent years, an increasing number of cases, mostly due to genotype 3 or 4 HEV, have been recognized. These are more often elderly men who have other coexisting illnesses, and appear to be related to zoonotic transmission from pigs, wild boars and deer, either food-borne or otherwise. in addition, chronic infection with genotype 3 HEV has been reported among immunosuppressed persons in these regions. A subunit vaccine has been shown to be effective in preventing clinical disease, but is not yet commercially available (Aggarwal and Naik, 2009).

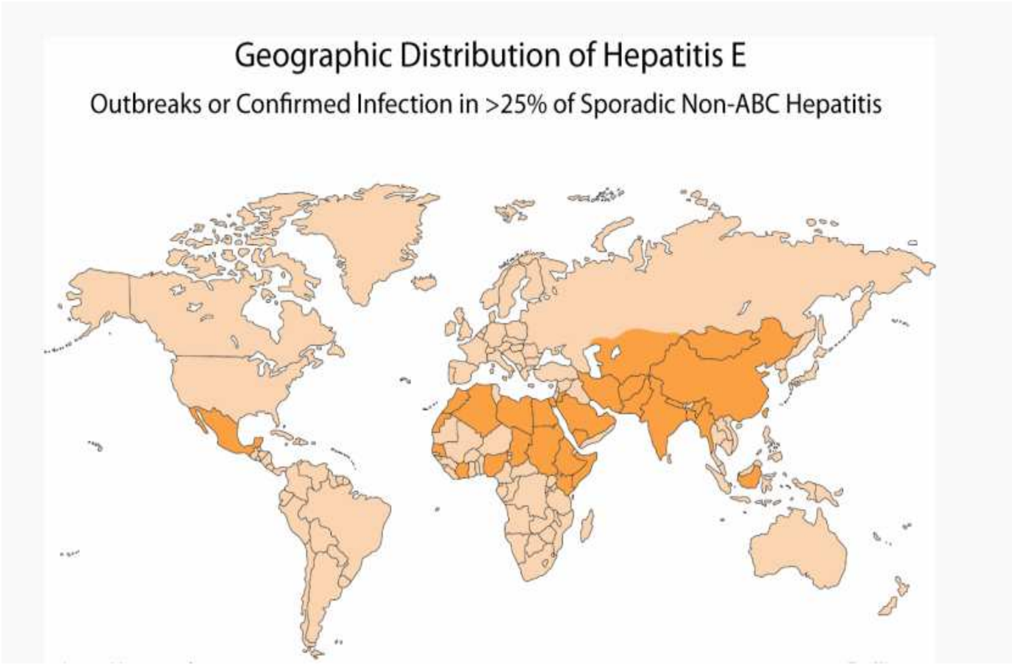

Figure 1-4. Ggeographic distribution of Hepatitis E

HEV in Endemic Regions

The issue regarding existence of an animal reservoir in endemic regions remained uncertain. The zoonotic hypothesis for transmission of HEV is based mainly on 3 main findings:

- High prevalence of anti-HEV antibodies in several animal species
- Isolation of HEV genomic sequences from pigs.
- Genomic sequence homology between human and animal HEV isolates.

Most of the supporting genomic data were collected from non-endemic regions. In contrast, data from endemic regions are sometimes conflicting. For instance isolates from animals and sporadic human cases have belonged to the same genotype (genotype 4) in Vietnam and China. In India animal isolates were all belonged to genotype 4 and human isolates to genotype 1 HEV, which is responsible for the large majority of cases in all endemic countries, has never been isolated from pigs. Moreover in experimental studies, genotype 1 virus is unable to infect pigs.

Thus, zoonotic transmission may not be a major mode of spread in these areas, in particular for the widely prevalent genotype 1 HEV. It also appears that in the regions where hepatitis E is endemic, the infection is acquired from either an environmental or a human reservoir through poor general sanitation, contaminated drinking water supplies and lack of attention to personal hygiene. Further data are needed before zoonotic transmission of HEV can be implicated in these regions.

HEV in Non-Endemic Regions

Different studies have shown that in non-endemic regions, where outbreaks have not been reported, and the disease accounts for only a minority of reported cases of acute viral hepatitis. Meanwhile, in recent years, solitary cases or small series of cases related to autochthonous transmission of hepatitis E in these regions have been recorded in the USA, Europe (including UK, France, the Netherlands, Austria, Spain and Greece), and developed countries of Asia–Pacific (Japan, Taiwan, Hong Kong, Australia).

The mode of transmission in most HEV cases could not be identified, although zoonotic spread has been anticipated. Zoonotic spread of the virus was first suspected when genomic sequences of HEV isolates from two autochthonous cases in the USA were found to be more closely related to swine HEV than to human HEV isolates (Meng 2010; Pavio *et al.,* 2010). This was supported by experimental cross-species transmission of human isolates to pigs, and of swine HEV to primates. The most direct evidence for zoonotic transmission was provided by a cluster of Japanese cases in those who had consumed inadequately cooked deer meat a few weeks prior to the commencement of illness (Tei *et al.,* 2004; Takahashi *et al.,* 2004; Pelosi and Clarke 2008).

The genomic sequences of HEV isolated from these cases were identical to those from the leftover frozen meat, establishing foodborne transmission beyond doubt. In another case in Japan, wild boar meat was similarly implicated. In addition, genomic sequences from the deer meat had a high (99.7%) sequence homology with HEV isolates from a wild boar and another wild deer from the same forest, suggesting transmission between these animal species and from deer to humans. Since then, several evidences have supported existence of zoonotic transmission in non-endemic regions. In one study, seven of 363 commercial packets of pig liver sold in Japanese grocery stores were found to contain genotype 3 or 4 HEV(Aggarwal and Naik, 2009; Scobie and Dalton, 2013). These isolates shared a high degree of identity with HEV isolate from a previous human case. In the USA also, genotype 3 HEV RNA was isolated from 14 of 127 commercial pig liver packages (Feagins *et al.,* 2007). Furthermore, a large proportion of Japanese patients with sporadic hepatitis E admitted to have eaten uncooked or undercooked pig livers (Yazaki *et al.,* 2003).

A case–control study among patients with autochthonous hepatitis E in the UK, however, failed to show any association with consumption of pig meat or contact with pigs. Contaminated shellfish were shown in some studies as a mode of transmission (Aggarwal and Naik, 2009; Lewis *et al*, 2010).

HEV Seroprevalence data

Hepatitis E serology

The immune response to hepatitis E infection is similar to hepatitis A infection. As shown in Figure 1-5, IgM anti HEV appear and rise sharply about 40 days following exposure and IgG appears immediately after IgM and peaks 2-3 weeks later or when about symptoms start to subside. The IgM titer decreases after several months while IgG generally remains detectable for longer periods (1-4 years), depending upon the assays used. Anti-HEV antibodies have been found in healthy subjects living in all geographical areas, although the prevalence varies widely. In general, prevalence rates are higher in developing countries where hepatitis E is common than in countries where clinical cases due to hepatitis E are uncommon.

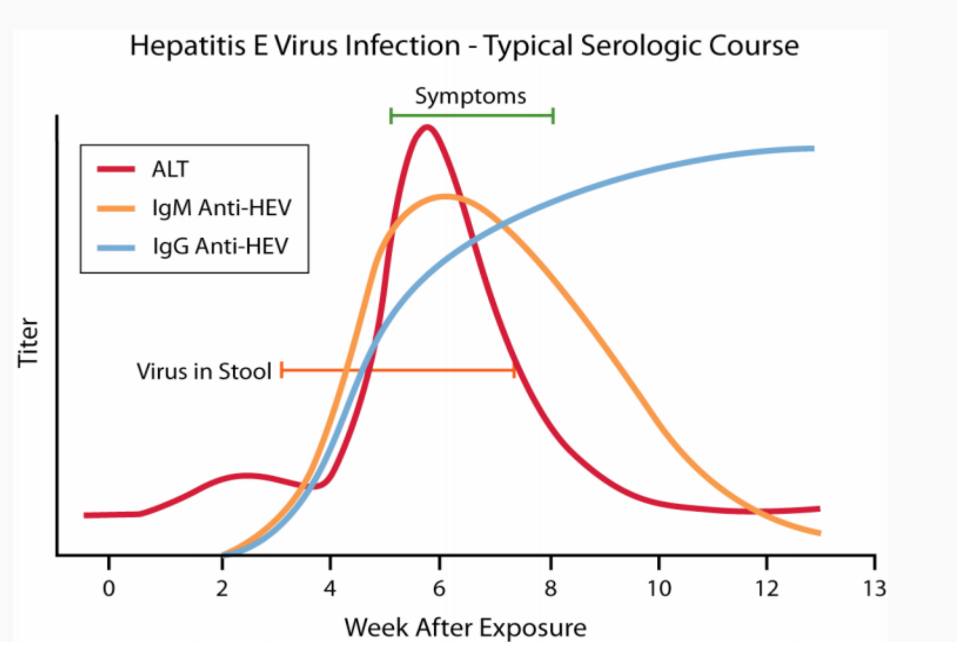

Figure 1-5. Serologic events following Hepatitis E infection

In India and other endemic countries, despite common occurrence of clinical cases and outbreaks of hepatitis E, the age-specific seroprevalence rates of anti-HEV are much lower than those for hepatitis A virus (HAV) and other enterically transmitted infections, such as Helicobacter pylori**.** In developed countries, anti-HEV antibody prevalence rates ranging from 1% to above 20% have been reported (**Table1-5**) these appear to be higher than those expected from the low rate of clinically evident hepatitis E disease in these areas.

Some of these variations may be related to differences in the assays used. In a direct comparison using a panel of coded sera, various anti-HEV antibody assays showed sensitivity rates varying from 17% to 100%. A comparison between 2 types of assays that have been used for the detection of anti HEV antibodies in seroprevalence studies is shown in **Table 1-4.**

Table 1-4. Comparison of the results obtained with two different HEV IgG kits.

Subjects	Author/ Year	Country	Assay 1		Assay 2	
			No	%	No	%
Blood donors	Zanetti *et al*, 2011	Italy	13/433	3	72/433	16.6
Blood donors	Bendall *et al*, 2010	UK	18/500	3.6	81/500	16.2
Blood donors	Mansuy *et al*, 2004	France	88/529	16.6	268/512	52.5
Healthy individuals	Park *et al*, 2012	Korea	21/147	14.3	34/147	23.1

(Refs: Zanetti *et al*, unpublished data, 2011; Bendal *et al*, 2010; Mansuy *et al*, 2011; Park *et al*, 2012.)

In developed countries, veterinarians and swine farm workers who come in close contact with pigs have higher anti-HEV seroprevalence rates than the general population. Studies in the USA, Spain, and Sweden indicated 4.5, 5.4 and 1.4 times higher anti IgG titres between exposure and non exposed groups respectively (Withers, 2002; Galiana, 2008; Olsen, 2006). Studies in Western Europe and United States indicated that presence of antibody to HEV (anti-HEV) is more common than expected in areas where HEV infection is not endemic. Some studies suggest that HEV infection maybe an asymptomatic zoonotic infection in industrialized countries (Teo, 2006; Christensen *et al.*, 2002, Meng *et al.*, 1998).

The low rates of autochthonous cases of Hepatitis E in industrialized countries and also detection of HEV strains that are closely related to human strains in wild boars and pigs have led to assumption that some animal species may act as reservoirs in non endemic areas (Renou *et al*,. 2007). Galiana *et al.*, (2008) carried out a study for the purpose of investigating the prevalence of hepatitis E virus (HEV) and the risk factors for the acquisition of the virus in a population in contact with swine and unexposed to swine in Spain .Their resluts indicated that people exposed to swine were observed to be 5.4 times ($P < 0.03$) at risk of having anti-HEV IgG comparing to control group and HEV infection should be treated as a occupational illness in swine workers.

In brief HEV has been suggested to be a zoonotic infection where pigs may be an important reservoir for the disease and also specific swine strains of HEV have been identified which can infect humans.

In Europe, the prevalence of HEV-antibodies in human sera ranges from 1% to 5%, and in Italy HEV infection accounts for approximately 10% of the cases of acute non-A, non-B, non-C viral hepatitis. Most of Hepatitis E cases in Italy are due to travels in endemic regions, but in 1999 a HEV strain was isolated from a patient who had not travelled in endemic regions. This isolate was genetically different from those isolated in other countries and resulted the first autochthonous Italian human HEV strain (La Rosa *et al.*, 2011).

Table 1-5. The Prevalence of anti HEV antibodies among selected populations and animal species in developed countries.

Author	Publication Year	Country	Sample size	Anti HEV Antibodies (%)	Study	Lab Test	Description
Scotto *et al.*	2013	Italy	412 Immigrants (developing countries)	21.4	Cross sectional	EIA	*High circulation of HEV in the immigrant population. Anti-HEV IgM was found in 34/81 subjects (41.9%) The high prevalence of acute hepatitis mainly in subjects who arrived in Italy during the same period from the same countries (Eritrea, Ethiopia, and Somalia).*
Vulcano *et al.*	2007	Italy	Workers 92 General population 3511	3.3/ 2.9	Cross Sectional		*No significant difference in anti-HEV prevalence was observed between the two groups*
Withers *et al.*	2002	USA	Swine workers (n = 165) / non-swine workers (127)	10.9/2.4	Cross Sectional	ELISA	*swine-exposed subjects had a 4.5-fold higher antibody prevalence*
Meng *et al.*	2002	USA	468 Swine Vet (389US)/400 BD	26 /17	Cross Sectional	ElISA /AIgG	*97% concordance with human*
Galiana *et al.*	2008	Spain	198 (101 exp/ 97 unexp)	18.8/ 4.1	Cross Sectional	ELISA	*People exposed to swine were observed to be 5.4 times (P = 0.03) at risk of having anti-HEV IgG.*
Drobeniue *et al.	2001	Moldova	Swine Farmers/ Controls 264/255	51.1/ 24.7	Cross Sectional	EIA	*The increased prevalence of HEV infection among persons with occupational exposure to swine suggests animal-to-human transmission of this infection*
Olsen *et al.*	2006	Sweden	EXP (Pig Farmers)/Controls 1221/1130	13/9.3	Cross sectional	EIA	*No significant statistical difference*

***Moldova is an exception, being located in Europe but accounted as a developing country.**

Table 1-6. The Prevalence of anti HEV antibodies among selected populations in developing countries.

Author	Year	Country	Sample size		HEV Prev (%)	Method	Lab Test	Description
Wibawa ***et al.***	2004	Indonesia	Healthy individuals (BD)1115		**20**	Cross Sectional	ELISA/RT	*Presumably indigenous HEV strain(s) is circulating in Bali, Indonesia and that HEV infection may occur via zoonosis even in developing countries.*
Choi ***et al.***	2003	Korea	Blood Donors	96	17.7	Cross sectional	ELISA	*Three new swine HEV isolates (gt 3) were identified.in 2- to 3-month-old pigs in Korea.*
Lin ***et al.***	2000	Taiwan	Healthy subjects	271	11	Comp. Cross sectional	ELISA RT-PCR	*Sensitivity IgG/ IgM 86.7/53.3* *Specificity IgG/ IgM 92.1/ 98.6*
Pourpongporn ***et al.***	2009	Thailand	Swine workers Poultry workers Government fficers	168 102 138	27.2 24.5 16.7	Cross sectional	ElISA	*exposure and reinfection to HEV are higher in farmers than that in government officers. Poor environmental conditions in farms, occupation and low socioeconomic status might be risk factors in HEV infection.*

Leptospirosis

Leptospirosis is a zoonotic infection that can cause a variety of different clinical pictures in human, from asymptomatic infection to severe multiorgan disease with hepatorenal failure (Weil's disease). It has a global distribution (except for the polar regions), but is mainly prevalent in the tropics. The taxonomy of leptospires (spirochaetes with hooked ends) is complex and still evolving. Historically, two phenotype-based species *Leptospira interrogans* (containing pathogenic strains) and *L.Bireflexa* (containing non-pathogenic strains) were further subdivided on the basis of serological tests into more than 250 serovars, with antigenically related serovars grouped into a smaller number of serogroups.

Each serovar has a defined host range, and the number of serovars present in any given geographical area tends to be restricted, particularly in temperate zones rodents and other small mammals are the main animal reservoir. They become infected in infancy, develop a chronic renal infection and excrete leptospires in their urine during their life. Excreted organisms might remain viable in water or soil for weeks. Larger mammals, such as cattle, dogs, and pigs, develop a symptomatic infection that may be fatal, or become chronically infected and shed leptospires into the environment. People who have direct contact with animals, water or soil are at greatest risk of occupationally acquired leptospirosis**,** which was first described in miners in the early twentieth century, although jaundice in rice-paddy workers has been recognized since ancient times in China. Infection is seasonal, with a high incidence in the rainy season in the tropics, and in the summer or autumn in countries with temperate climates. Infection rates may increase after heavy rainfall or flooding.

Occupational risk groups include veterinarians, dairy and pig farmers, abattoir workers, hunters and trappers, fish workers and fish farmers, rodent control workers, sewage and canal workers, sugar cane cutters, banana farmers, and the military. Leptospires invade through mucous membranes, conjunctivae or microabraded or water-sodden skin. They are disseminated via the bloodstream and are therefore widely distributed throughout the body (Miller and Heptonstall, 2010).

Sporadic leptospirosis is linked with human contact with contaminated environments in various settings: on the job (veterinary, sewer, and slaughterhouse workers), in poorly hygienic inner-city alleys and slums, during adventure travel and other non-work-related outdoor activities, and during military training exercises in endemic regions.Reliable data on the incidence of leptospirosis and on rates of associated morbidity and mortality remain scant (Vinetz, 2008).

The number of human cases worldwide is not well-documented. According to WHO, it probably ranges from 0.1 to 1 per 100 000 per year in temperate climates to 10 or more per 100 000 per year in the humid tropics. During outbreaks and in high-risk groups, 100 or more per 100 000 may be infected. In Brazil 4128 cases were recorded in 2000, according to the National Foundation of Health (WHO and ILS, 2003).

The recorded history of human leptospirosis in Italy begins in 1917 when Moreschi reported the disease in 17.5% of hospitalized soldiers from the Isonzo front . Until the early sixties, leptospirosis was a relatively widespread occupational infection, especially in rice-fields workers, with a morbidity rate from 3000 to 12,000 cases per year. In the following years, with the mechanization of rice cultivation and the vaccine-prophylaxis measures for the subjects at risk, there was a significant fall in the number of cases, the epidemiological pattern changed, and now there are other groups at risk.

Today, farmers and agricultural workers are the main occupational group at risk but there are frequent reports of leptospirosis contracted during recreational activities and acci-dental events. Despite its low incidence at present, the likelihood of sudden common-source outbreaks shows that the problem leptospirosis must not be underestimated. In Italy, during the period of July 1984, a waterborne outbreak with 33 cases of leptospirosis, 3 of which fatal, occurred in a small town in Marche, a region in central Italy where the illness is seldom identified (Ciceroni *et al.*, 2000).

Until the 1980s, the Veneto region (North-East Italy) and the Vicenza area, in particular, were characterised by high endemism for leptospiral infection. In the following figures human leptospirosis in the Vicenza area (Italy) from 1990 to 2003 are shown (results of the study conducted by Conti *et al.*, 2005).

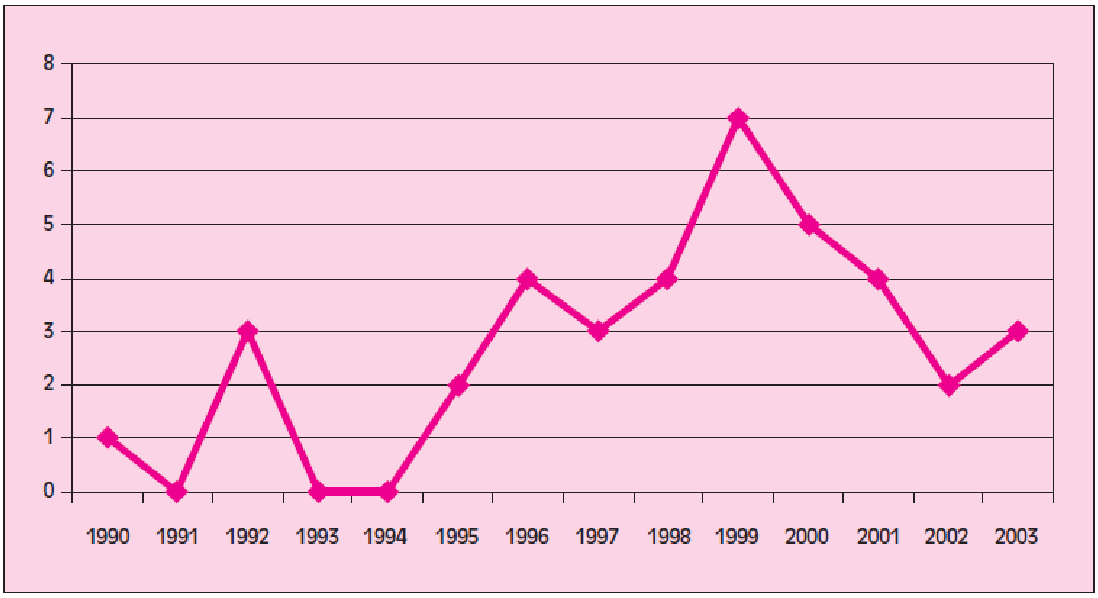

Figure 1-6. Number of cases of leptospirosis annually, 1990- 2003 in Italy (n = 38). (Ref Conti et al., 2005)

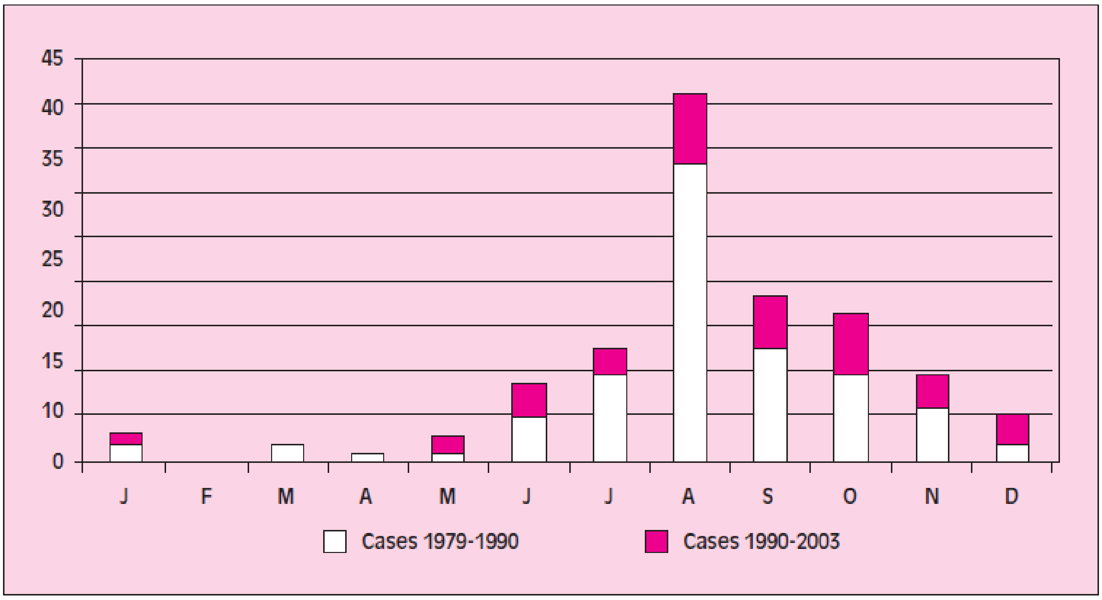

Figure 1-7. Comparison of monthly incidence from 1979 to 2003 in Italy (n = 124). (Ref Conti et al., 2005)

Q Fever

Q fever is a zoonotic disease caused by Coxiella burnetii, a species of bacteria that has a global distribution. Q fever is a widespread zoonotic infection that can affect various birds and mammals. Domestic herbivores (sheep, cattle and goats) are the main source of human infection; the main occupational groups at risk are vets, agricultural workers, meat-processing workers and research laboratory workers. Ticks, can be infected and therefore be responsible for maintaining the animal reservoir. Pet, cats, dogs and rodents may also be infected.

Animal infection is usually asymptomatic and chronic, but may cause abortion in cattle, sheep and goats. High concentrations of the organism are found in placenta, amniotic fluid and fetal membranes which may contain as much as 10^9 organisms/g. Birthing fluids may contaminate the surrounding environment, and, because the organism is relatively resistant to heat and to drying, it can survive in litter, bedding and dust for weeks. It can also be found in the milk, urine and faeces of infected animals, but inhalation of aerosolized organisms seems to be the most important method of spread to humans. The infective dose is low: fewer than ten organisms may be enough to produce clinical disease. In an outbreak in Switzerland, more than 400 people living along a road on which sheep descended from their mountain pastures became infected. In Briançon, France airborne infection from exposure to a sheep slaughterhouse, where aerosols of contaminated dust were created by helicopters flying over from a nearby heliport caused an outbreak. More recently, widespread community outbreaks, associated with large-scale goat farming, have been reported from the Netherlands.

In human, many infections are asymptomatic (54 percent in the Swiss outbreak). The main features of clinically apparent infection are an acute pneumonic syndrome (one of the causes of the so-called ‘atypical pneumonias’), a non-specific febrile illness or acute hepatitis. The incubation period following exposure is about two weeks (mean, 15 days; range, 2–41 days). Headache and myalgia are prominent features in the pneumonic and non-pneumonic forms of illness. In addition to hepatitis, the major manifestation of chronic Q fever is endocarditis. Endocarditis is often a delayed diagnosis and it is common for patients to have the more chronic features of endocarditis, such as finger clubbing and splenomegaly, together with features of immune complex activation. It is vital to take a careful history about occupational or other exposure (Miller and Heptonstall 2010; CDC website).

Lyme Disease

Lyme disease is the most common tick-borne infection in the northern hemisphere. Transmission by an arthropod vector was postulated and in 1982 the causative organism was isolated both from ticks and humans. The main species responsible for Lyme disease is *Borrelia burgdorferi* sensu strictu, although in Europe the condition may be caused by two closely related organisms, *B. garinii* and *B. afzelii*. The bacterium is transmitted by hard ticks of the genus *Ixodes*. Forest rodents and small mammals, such as deer, are the usual animal hosts. Man can be infected by the bite of an infected tick (usually the nymph stage). Lyme disease occurs wherever there is the pool of infected mammals and the suitable hard ticks to maintain transmission.

The risk of Lyme disease would seem to be an important concern to outdoor workers in endemic areas. To date, Lyme disease has been documented in many occupational groups, including forestry workers, farmers, veterinarians, military recruits, orienteers, and outdoor workers in general. The disease is mainly reported from the United States and Europe. In 2008, nearly 28,921 cases of Lyme disease were reported to the Centre for Disease Control and Prevention. In the United States, more than 90 percent of cases are reported from the northeastern states with just a few reports from the southwest. It also occurs in the United Kingdom, but is mostly reported from other European countries, particularly Germany, Austria, Slovenia and Sweden, usually from rural areas that have the basic mammal and tick populations. The distribution of Lyme disease outside Europe and North America is unclear: there is controversy about whether infection occurs in Australia, and few data for Africa, Asia or Latin America. (Miller and Heptonstall, 2010; CDC website).

Lyme disease has been documented in many occupational groups, including forestry workers, farmers, veterinarians, military recruits, orienteers, and outdoor workers in general. According to WHO report (LB in EU 2006) there was a decreasing trend of LB incidence from south to north in Scandinavia and from north to south in Italy, Spain and Greece. The highest incidences of LB in Europe were found in the Baltic States and Sweden in the north, and in Austria, the Czech Republic, Germany, Slovenia and central

Europe. Various evidences suggest that Lyme disease, caused by *Borrelia burgdorferi* sensu lato, is endemic in different areas of Italy and the main vector of Lyme disease in Italy is the hard tick *Ixodes ricinus*, a species widespread in mountain regions populated by wild ungulates. People who live in these areas and also forestry workers are accounted as at risk of contracting Lyme disease.

Nuti *et al* (1993) carried out a seroepidemiologic survey from 1987-1991 for antibodies to leptospira, and Borrelia in selected Italian population groups. In the mountainous areas of northeastern Italy, the prevalence of antibody to *L. bratislava*, and *L.saxkoening* serotypes, was observed in 10-12% of the farmers and forestry workers. Only a few sporadic clinical cases of leptospirosis have been reported from these regions. Antibodies to *Borrelia burgdorferi* (by IFA) were observed in 19% of the rangers and forestry workers, with lower values in farmers (10%) and hunters (8%). These data propose the presence of a large number of asymptomatic infections with *B.burgdorferi* and the leptospires in the densely wooded areas of the Alpine Italian regions.

Other study by Ciceroni and Ciarrocchi (1998) indicitaed that in contrast to high prevalence of antibodies to *B. burgdorferi sensu lato* in the groups at risk (up to 27.2% for forest workers), the seroprevalence of the healthy population in general was lower.

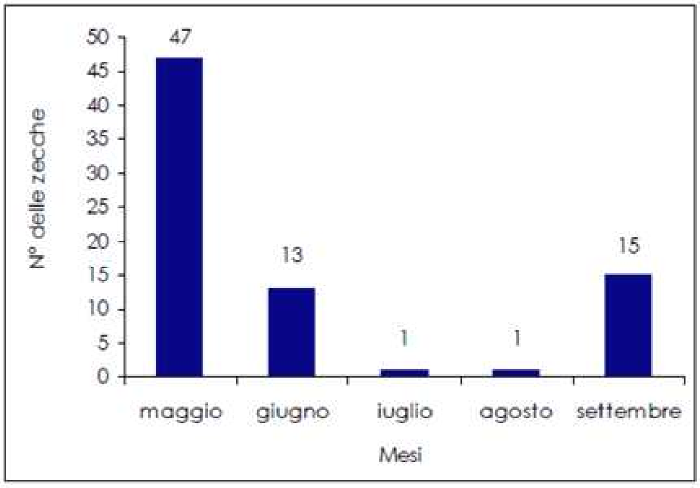

Fig 1-8. Monthly distribution of the vector in a study of central Italy (Marche Region; Pascucci and Cammà, 2010)

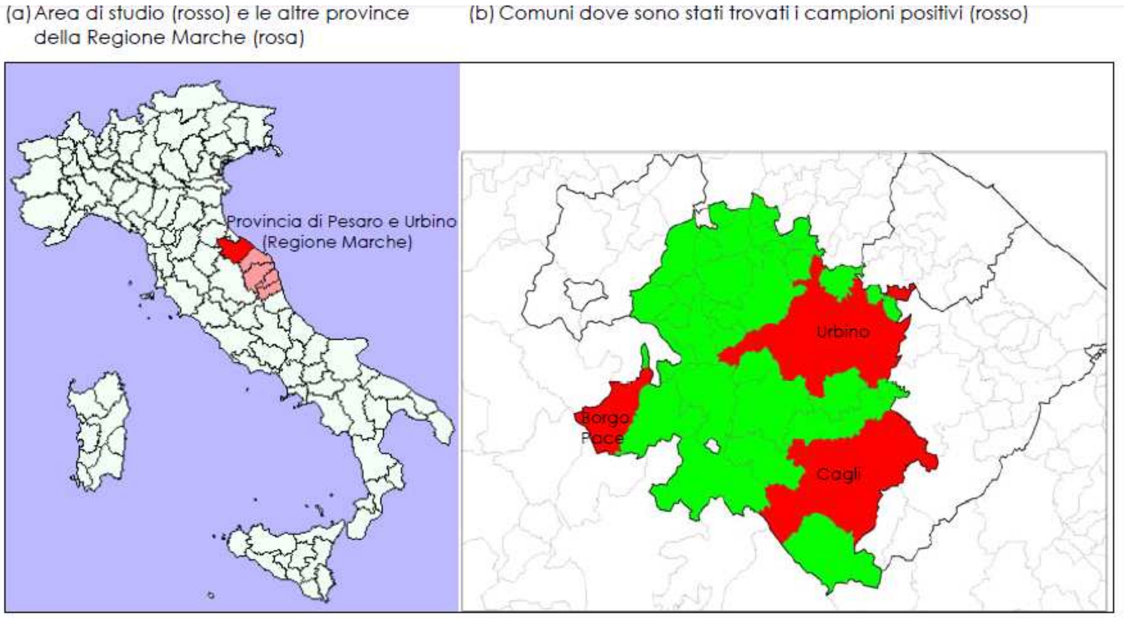

Fig 1-9. Study area and the cities that positive cases were reported (Pascucci and Cammà, 2010)

Erisipelas

Erysipeloid (‘pork finger’, ‘whale finger’, ‘fish-handler’s disease’) is caused by the Gram-positive bacterium *Erysipelothrix rhusiopathiae*, that is widely distributed in nature and found in a wide range of vertebrates and invertebrates, including domestic swine (causing swine erysipelas), poultry sheep, shellfish and fish. Most human infections are occupationally acquired, by direct contact with infected fish, animals or their products; minor trauma (e.g. puncture wounds and cuts) facilitates transmission. Infection generally presents five days to two weeks after exposure as an extremely painful, well demarcated, slightly raised cellulitis on the finger or hand, centred on the site of inoculation, that is characteristically violaceous at the spreading edge with central clearing. Fever is rare. *E. rhusiopathiae* is a rare cause of endocarditis (usually of a native aortic valve); about 40 percent of cases have a recent history of an erysipeloid-like skin lesion, or a concurrent one (Miller and Heptonstall, 2010)

Brucellosis

Brucellae are facultative intracellular Gram-negative coccobacilli that generate the clinical condition known as ‘brucellosis’. There are at least eight named species, each closely associated with specific animal hosts, but *Brucella abortus*, *B. suis* and *B. melitensis* cause most human disease. *B. melitensis*, first isolated by Bruce in 1886, and later shown to be the cause of Malta fever, caused by drinking unpasteurized milk from infected goats, is found also in sheep and camels, and also in recent times emerged as a problem in cattle in southern Europe. *B. suis* was first isolated from aborted pigs, and is also found in wild boars and hogs, and cattle; one serovar is host adapted to reindeer and caribou. *B. abortus* was shown by Bang to cause abortion in cattle, and also occurs in buffalo, bison and elk. The other species are either rarely (*B. canis, B. maris*) or never *(B. neotomae)* linked with human disease.

Nearly all human cases of brucellosis are acquired directly or indirectly from animals, and disease incidence in humans reflects the prevalence of infection in local domestic animal herds. Infection is found worldwide, but is especially prevalent in the Mediterranean countries, the Middle East, South Asia, Africa and parts of Latin America. Humans acquire infection by direct contact with infected animals, or their birthing or abortion products (when the organism gains entry through cut or abraded skin, or conjunctivae or mucous membranes) or by inhalation of aerosolized animal material.

Infection can also follow ingestion of raw milk or unpasteurized dairy products. Human-to-human spread by sexual contact has been described, but is uncommon. Test and slaughter policies combined with vaccination programmes have led to the near eradication of *B. abortus* in cattle in the United States, United Kingdom and other industrialized countries, and this has been paralleled by a decline in the incidence of cases in humans. Programmes to control *B. melitensis* have been less effective. Most of countries in Northern Europe are animal brucellosis free, although infections may occur in returning migrants occupationally exposed abroad, in travelers who have eaten unpasteurized dairy products, and in older or retired workers infected when animal disease was still common. Brucellosis is an occupational hazard for agricultural workers, abattoir workers, veterinary workers and meat packers, hunters and laboratory personnel (Miller and Heptonstall 2010).

Streptococcus suis infection

Streptococcus suis is a swine pathogen with a universal distribution, found wherever pigs are farmed. It causes meningitis, septicaemia, arthritis and meningitis in pigs; disease is more common when pigs are intensively farmed in suboptimal conditions. Human infection is linked with direct contact with sick or carrier pigs, and with eating undercooked pork products (mainly traditional foods, e.g. pig’s blood), so there is an occupational risk for pig farmers (including ‘backyard farmers’), slaughterers, abattoir workers, butchers and veterinarians. Wild boar hunters are also be at risk. Human infection is usually caused by *S. suis* serotype 2. Infection is uncommon and sporadic in the United States and the United Kingdom, but the organism has recently been recognized as an important zoonotic pathogen in China and Southeast Asia. In 2005, it caused an outbreak of streptococcal toxic shock syndrome (STSS) and meningitis that affected 204 people (with 38 deaths), mostly men who had slaughtered or eaten sick pigs; recent surveys suggest that *S. suis* is a common cause of acute bacterial meningitis in adults in Vietnam, Thailand, and Hong Kong.

The infection has a short incubation period (from a few hours to three days); cases present with clinical features of meningitis (headache, fever, neck stiffness, photophobia, nausea or vomiting) or septicaemia, and additionally may have signs of STSS, including purpura or a haemorrhagic rash. Endocarditis and septic arthritis may also occur. Diagnosis is made by culture of cerebrospinal fluid (CSF) and blood and; a PCR-based test is also available.. Mortality rates (particularly in STSS) may be high; deafness is a common outcome of meningitis. Prevention includes standard good hygiene (e.g. hand hygiene, using appropriate PPE during slaughtering) and ensuring that all pork products are well cooked before eating (Miller and Heptonstall 2010).

Salmonellosis

There are approximately 2000 serotypes of the *Salmonella* genus found in the environment, domestic and wild animals, and humans. Normally associated with contaminated food, the disease is often transmitted through hand-to-mouth contact from either ill or asymptomatic individuals or animals, surfaces they have contaminated, their bedding or water they have contaminated. A number of species kept as pets have been linked with human *Salmonella* infections. The CDC estimates that there are 40,000 cases of salmonellosis in the United States every year, but there is major underreporting because many of the milder cases are not identified or reported. They also estimate that 600 individuals die yearly (CDC website).

All clinically important salmonellae are now classified as a single species *Salmonella enterica*– with serotypes (now grouped within one of six subspecies), *Salmonella typhimurium*becomes *S. enteric* subsp. *Enteric* ser Typhimurium, or is shortened to

S.Typhimurium. Despite their microbiological similarities, there are major clinical and epidemiological distinctions between the so-called ‘typhoidal’ and ‘non-typhoidal’ salmonellae. The former group, which includes *S. Typhi* and *S.Paratyphi*, are exclusively human infections that are spread from person to person.

Infection is acquired by the faeco–oral route, but the major manifestations (at least in the early stages) of infection are due to bacteraemia rather than luminal infection within the gastrointestinal tract. By contrast, the non-typhoidal salmonellae are mainly zoonoses and having been acquired via the faeco–oral route, their initial pathogenesis is within the gut to produce ‘gastroenteritis’, although they may go on to produce invasive disease, with bacteraemia and subsequent localized infection.

Most infections are caused by ingestion of contaminated food or water, and are not occupationally acquired. Many cases are associated with overseas travel and several serotypes of salmonella can also be acquired from pet animals, including amphibians, and reptiles and the small rodents used to feed them. The main occupational groups at risk are veterinary and agricultural workers, and laboratory workers. Infection of workers in a vaccine production plant has also been reported. Food handlers may transmit infection; food safety and hygiene regulations cover employment and exclusion practices (Miller and Heptonstall 2010).

Tetanus

Tetanus which is a vaccine preventable disease still is significant health concern in the world. *Clostridium tetani*, the tetanus bacillus, is a spore-forming, anaerobic, gram-positive bacterium. Clinical disease is caused by a powerful neurotoxin produced by the vegetative state of the bacterium growing in contaminated wounds. *C. tetani* spores are ever-present in the environment and can be introduced into the body through nonintact skin, usually via injuries from contaminated objects. Lesions that are considered “tetanus prone” are wounds contaminated with dirt, saliva or feces; burns; punctures; crush injuries; or injuries with necrotic tissue. However, tetanus has also been linked with apparently clean superficial wounds, insect bites, surgical procedures dental infections, chronic sores and infections, compound fractures and intravenous drug use.

In 2006, an estimated 290,000 people worldwide died of tetanus, most of them in Asia, Africa, and South America. Worldwide, the disease is more common in agricultural settings and in areas where contact with soil or animal excreta is more likely and immunization is inadequate. In developing countries, tetanus in neonates born to unvaccinated mothers (neonatal tetanus) is the most common form of the disease.

Non vaccinated or inadequately vaccinated people are at risk when they are injured by a contaminated object, require surgery or dental care in unhygienic conditions use or injection drugs. Acute manifestations of tetanus are characterized by muscle rigidity and painful spasms, often starting in the muscles of the jaw and neck. Severe tetanus can lead to respiratory failure and death. The incubation period is usually 3–21 days (average 10 days), although it may range from 1 day to several months, depending on the character, extent, and location of the wound. Most cases occur within 14 days. In general, shorter incubation periods are associated with more heavily contaminated wounds, more severe disease, and a worse prognosis (CDC Website).

Farmers are at high risk for injury, the primary factor associated with tetanus. It was reported that farmers acquire tetanus at a higher rate than other population despite the fact that no difference in immunity to tetanus has been reported between rural and urban dwellers in large epidemiological studies (Hayney *et al*., 2003). This data seems to suggest the higher risk present in rural areas. Tetanus is a rare disease in the developed countries. In 2007, a total of 28 cases were reported to the U.S. national surveillance system. Most cases occur in unvaccinated or incompletely vaccinated individuals. Persons >60 years of age are at greater risk of tetanus because antibody levels decrease over time. Injection drug users particularly those injecting heroin subcutaneously ("skin-popping") are increasingly recognized as a high-risk group (Thwaites and Yen, 2012).

In Italy, tetanus vaccination became mandatory for military recruits in 1938. In 1963, recommendations were extended to children during the second year of life and selected occupational groups considered to be at risk groups (e.g., agricultural workers and animal breeders). In 1967, the recommended age of childhood vaccination was lowered; the current vaccine schedule calls for the three doses to be administered at 3, 5, and 11-12 months of age, followed by booster doses at ages of 5-6, 11-15 years, and every 10 years thereafter. Tetanus reporting has been mandatory in Italy since 1995. It is considered a Class I condition, that requires notification within 12 hours of a suspected case (Epi Centro, 2002).

According to ECDC In 2009, 128 cases of tetanus were reported by 25 EU and EEA / EFTA countries. The overall confirmed case rate remains very low at 0.02 per 100 000 population and the highest rate was reported by Italy (0.10 per 100 000). The confirmed case rate for tetanus remains very low in the EU, and the number of reported cases shows a slightly decreasing trend over the last few years (ECDC report 2011).

The incidence of reported tetanus in Italy decreased from 0.5/100,000 in the 1970s to 0.2/100,000 in the 1990 s. During this period of time, the case-fatality ratio decreased from 68% to 39%. Elderly women are the most affected: the proportion of women aged over 64 years among cases has increased from 60% in the 1970s to 76% in the 1990s (Pedalino, 2002).

MRSA Infection

Methicillin-resistant *Staphylococcus aureus* (MRSA) is generally resistant to beta-lactam antimicrobials, such as all penicillins, cephalosporins and carbapenems. MRSA colonisation in production animals detected in recent years has in several cases resulted in infections in humans, and infections with livestock-associated strain of MRSA may today be considered as a zoonosis. Pigs, in particular, have been considered as an important source of colonisation with livestock-associated MRSA in pig farmers, veterinarians, and their families, through direct or indirect contact with pigs.

In order to increase awareness and to assess the occurrence of MRSA in pig primary production across the EU, an EU-wide baseline survey was performed in 2008 to obtain comparable preliminary data on the occurrence and diversity of MRSA in pig primary production in all Member States through an organised sampling scheme. MRSA has since been detected in cattle, horses, chickens, pigs, rabbits, cats, dogs, seals and birds. An assessment of the public health implication of MRSA in animals and food was issued by the European Food Safety Authority in 2009**.**

The primary route of zoonotic transmission of MRSA is considered to be the direct or indirect occupational contact of livestock professionals with colonised animals, while the role of food as a source of human infection is considered to be low. Monitoring in primary production, including at slaughter, is crucial because of the main transmission route, while additional monitoring in food may help with the assessment of consumers' exposure via this route. A constant monitoring in fattening pigs, broiler flocks and dairy cattle, as well as in veal calves under 1 year of age and fattening turkey flocks, in those countries where production exceeds 10 million tonnes slaughtered/ year, is recommended every third year on a rotating basis. It is proposed that breeding poultry flocks and breeding pigs, as well as meat and raw milk products, are monitored on a voluntary basis. Representative sampling should be made within the framework of the national *Salmonella* control programmes for the poultry populations targeted, at the slaughterhouse for calves and either on farm or at the slaughterhouse for fattening pigs (EFSA Journal, 2012).

ECDC Report Regarding Zoonoses in the EU

According to the European Centre for Disease Prevention and Control (ECDC) annual epidemiological report (2011) Campylobacteriosis remains the most commonly reported infectious gastrointestinal disease in EU and EEA/EFTA countries, while the rate of confirmed cases of human Campylobacteriosis was stable during 2006–09. Meanwhile, there has been a steady decrease in the EU confirmed case rates for Salmonellosis over the last four years, although it continues to be one of the most common gastrointestinal infections in the EU/EEA. The reported case rates are very high in children, in particular in the 0–4 year-olds (124 per 100 000 population).

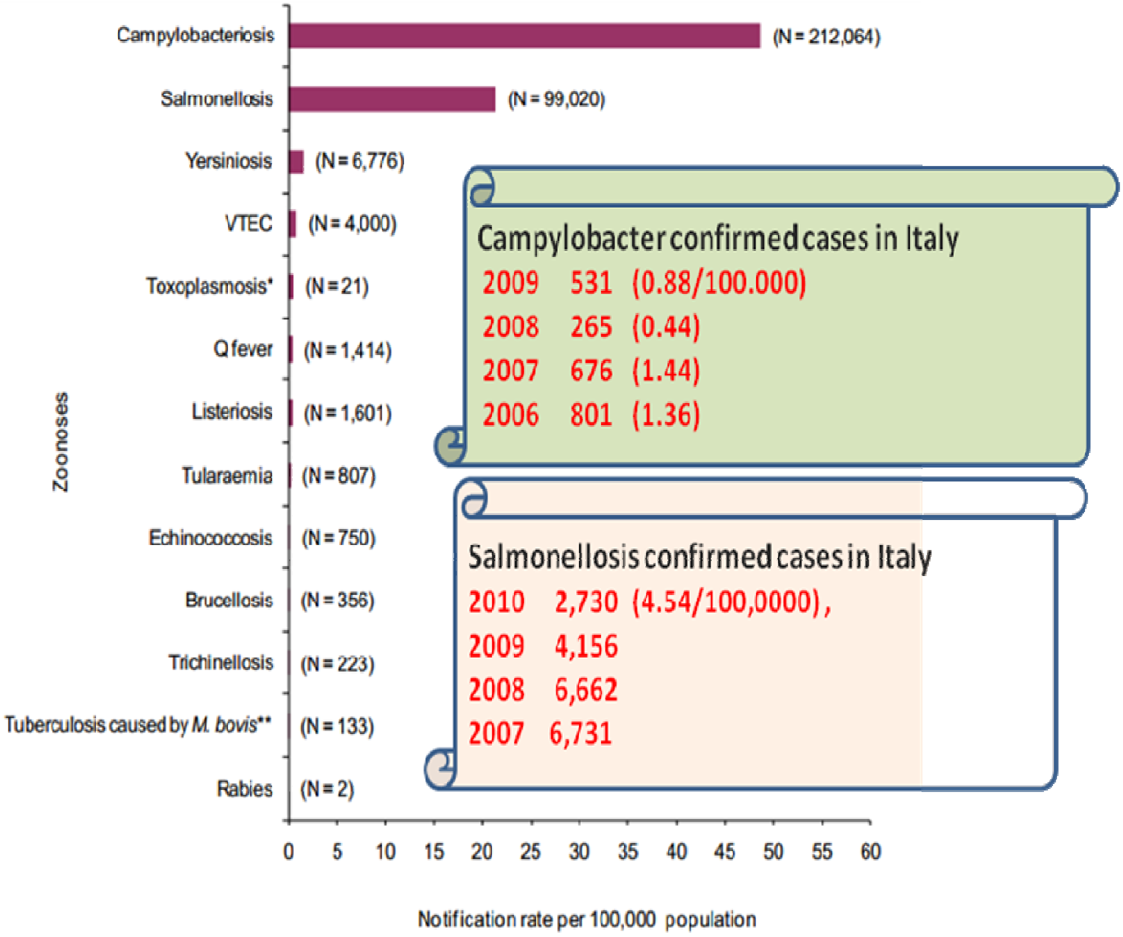

Fig 1-10. Reported notification rates of zoonoses in confirmed human cases in the EU, 2010.

1.4.0 Respiratory Diseases in Agriculture

The agriculture workplace has long been known to be linked with respiratory disease. Respiratory disease is among the most important chronic health conditions that affecting farmers Those who are at possible risk include farmers and farm families, abattoir workers agricultural workers, greenhouse and nursery workers, veterinarians, and grain elevator workers. Although the massive exposures leading to severe acute disease have decreased, it was known that there is a significant increase in subacute and chronic respiratory disease resulting from increased indoor air exposure (Donham *et al.,* 2000: Kirkhorn SR).

Animal breeders or dairy workers may spend about 40-50 hours or more than a week indoors in larger operations that can result in longer exposures to higher levels of gases and dusts. There are a number of common exposures that may result in respiratory illnesses, often with overlapping clinical signs and symptoms. These include organic dusts, bacteria, molds and gases from fermentation of silage and manure. Other respiratory hazards include inorganic dusts, pesticides, and other agricultural chemicals. There are also infectious respiratory conditions that are not unique to agriculture but may be encountered in the work and living environments associated with farm families.
(Kirkhorn, SR)

Asthma and Occupational Asthma

Asthma is a classic IgE antigen-antibody mediated sensitization to an environmental antigen and is defined as a chronic inflammatory pulmonary disorder with reversible obstruction of the lungs as a result of exposure to variable stimuli. The obstruction may reverse either spontaneously or with treatment. The clinical hallmarks are wheezing, cough, and dyspnea (air hunger).

Occupational asthma is the most common form of occupational lung disease. Occupational asthma (also known as work-related asthma) is asthma that is caused or made worse by exposures in the workplace. Estimates suggest that 15 to 23 percent of new asthma cases in adults are work related. The most common cause is from environmental allergens. Generally, farmers and agricultural workers have a lower prevalence of asthma than the general population. This may be because of the healthy worker effect in which those who do not tolerate the dusty work conditions leave that occupation (Kirkhorn SR).

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) remains a major public health concern. It is the fourth leading cause of chronic morbidity and mortality in the United States, and is predicted to rank fifth in 2020 in burden of disease worldwide, according to a study published by the World Bank/World Health Organization (Rabe *et al.*, 2007). COPD is a common inflammatory illness of the airways characterized by airflow obstruction usually is not fully reversible. It is mainly caused by smoking, but other factors including exposure to biological agents can play a significant role in its development. Livestock farmers have an increased risk of chronic bronchitis, COPD, and reduced forced expiratory volume (FEV1). COPD in farmers working inside confinement buildings is related to organic dust exposure and may become severe (Szczyrek *et al.*, 2011). The mechanism of this ‘‘farming- induced COPD’’ (FI-COPD) is not clear. Farmers are exposed to a wide range of organic and inorganic dusts and gases (Linaker and Smedley, 2002).

Several exposure elements, including dust, ammonia, and endotoxin, have been associated with respiratory symptoms in farmers (Donham *et al.*, 2000). Farmers do not seem to complain about respiratory symptoms until advanced stages of the disease. It was indicated that allergens found in the working environment could be transfered to the living environment of the farmer. Poor ventilation as well as high temperatures inside the animal buildings was shown to have a negative impact on respiratory symptoms and lung function parameters (May *et al*., 2012; Radon *et al*., 2003).

Extrinsic Allergic Alveolitis

Allergic alveolitis, also known as extrinsic allergic alveolitis and hypersensitivity pneumonitis, is an inflammatory disease involving the distal proportions of the airways. The disease has an immunological component, but it has been difficult to find the immunological mechanism by which antigens create granulomatous lymphocytic inflammation in the alveoli and bordering regions. The Institute of Medicine (2004) concluded that clinically significant allergic alveolitis occurred only in susceptible people exposed to sensitizing agents. The studies have shown that there is sufficient evidence of an association between the presence of mould and bacteria in damp indoor environments and allergic alveolitis.

Organic Dust & Immune system activation in Agricultural Workers

In addition to microorganisms, occupational exposure in animal breeding farms might also result in contact to other biological risk factors, in particular organic dusts. In general, the air in livestock buildings contains a large variety of microorganisms, gases and considerable amount of dusts which can remain suspended in the air for long periods and can therefore be inhaled (Simpson *et al.*, 1998; Rushton *et al* 2006). Dusts are characterized by a heterogeneous composition, containing endotoxin, lectins, pollens, feeds and antibiotic residues. There is strong epidemiological evidence that organic dusts and bacteria can cause infectious and allergic diseases both in animals and farm workers (Charavaryamath and Singh, 2006).

High exposure to organic dusts, especially when contaminated with large amounts of endotoxin, may result in a flu-like disease, with symptoms such as fever, chills, dry cough, malaise, mild dyspnea, headache and muscle pain, the so called "organic dust toxic syndrome (ODTS)" (Malmberg *et al.*, 1993; Wang *et al.*, 1997). The condition, which usually resolves within 72 hours, should not be confused with the toxic effects of gases produced by fermentation. Pig farming exposes workers to a mixture of organic dusts and high levels of endotoxins, ammonia, and the types of disinfectants in regular use that can contain respiratory sensitizers (Cullinan and Newman Taylor, 2010).

Also in subjects without any sign of overt diseases, inhalation of organic dusts might results in slight changes, indicative of immune system activation such as increase in blood cell count, IL6 and TNFα observed particularly in winter, when ventilation is reduced (Sandström *et al.*, 1992; Bønløkke *et al.*, 2009). It is unclear whether these changes represent an early adverse effect, able to evolve into overt diseases, or only adaptive and transient changes consequent to exposure to immune system activators. Studies addressing at the interactions between immune system and rural indoor environment, in particular animal breeding farms, are few and sparse, and no firm conclusions have been drawn from them (Larsson *et al.*, 1992; Palmberg *et al*, 2002).

Evidence suggests that among farmers, pig farmers are at higher risk of an asthma-like syndrome compared to farmers keeping other kinds of animals, while among plant farmers, greenhouse workers were at higher risk reporting symptoms of asthma. The prevalence of symptoms of allergies were significantly lower among animal farmers as compared to the population of the European Community Respiratory Health Survey (ECRHS website). Farmers with animal production had a significantly lower prevalence of allergic diseases than the general population. In contrast, the prevalence of chronic phlegm was higher in animal farmers. ODTS was also a major predictor of chronic bronchitis. It was indicated that allergens found in the working environment could be transferred to the living environment of the farmer. Poor ventilation as well as high temperatures inside the animal buildings was shown to have a negative impact on respiratory symptoms and lung function parameters. Results of an extensive European literature review reveal that, animal farmers are at high risk of chronic bronchitis. Intervention studies on the efficacy of different types of ventilation are needed. Furthermore, prospective studies on the association between ODTS and COPD should be done.

Serum Cytokines

Cytokines are humoral signaling molecules that attach to immune system cell membrane receptors at relatively low concentrations, generally acting over distances of a few cell diameters. Grossly elevated blood levels in states such as sepsis and cutaneous burns reflect the activation of the cytokine network associated with a concomitant deregulated immune response, but variations within physiologic ranges could also have significance. Notably, cytokine signaling operates in a pleiotropic (each cytokine acts on multiple molecular targets) and redundant (several cytokines respectively elicit the same cellular response) fashion. Thus, the blood levels of multiple cytokines collectively may reflect subtle states of immune dysfunction and/or immune-related disease. Collart *et al*. (1986) discussed that Monocyte derived cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-8 (IL-8) usually denominated as ‘‘proinflammatory’’ play an important role in local inflammation. Swine farmers have increased numbers of inflammatory cells, predominantly neutrophilic granulocytes, in the airways as assessed by bronchoalveolar lavage (BAL). It has previously been demonstrated in healthy subjects, that interleukin-6 (IL-6) and tumour necrosis factor-α

(TNF-α) increase in peripheral blood a few hours after swine dust exposure. In another study it was indicated that IL-8, which acts as a chemoattractant and activator of neutrophils at the site of inflammation, was markedly increased, and, therefore, was most likely involved in the recruitment of the neutrophils at the different sites. Meanwhile the lack of large increases in serum levels of TNF-α after swine environment exposure might be explained by the difference in sensitivity of the cytokine measurement kits that are used in the different studied (Cormier 2005).

1.5 The Internationl Centre for Rural Health (ICRH)

The data for this PhD research project have been gathered thorough health surveillance activities by ICRH, for this reason a brief information is explained in below.

ICRH is a WHO collaborating centre for occupational health that provides occupational health services to more than 1000 agricultural workers in about 400 enterprises located in the Lombardy Region. The main areas of activities include:

- Conducting an experimental activity of occupational health care provision to rural workers which is based on the key word “Bringing Occupational health care as close as possible to the places where rural people live and work”.

- Chemical and biological risk assessment at workplace and in general all the activities regarding to the workers’ Health and safety issues at work.

- Conducting several field studies in different agricultural enterprises, regarding chemical and biological risk assessments.

- Proposal submission and active participation in the research projects at national and international levels (e.g., a TEMPUS project addressed at rural health in Central Asia, in which the Centre acts as coordinator. The project has been granted by EU and the activities started in January 2013).

- Active participation in the preparation of criteria for diagnosis of the diseases listed in the new ILO list of occupational diseases, and link of this activity with the WHO one addressed at creating the forthcoming ICD 11 (The International Classification of Diseases 11th Revision).

- Interaction with the WHO-CC ICPS (Italy). Creation of a database for the collection of data coming from the activities of occupational health care of rural workers carried out by the Centre.

- Participation in national and International events related to Occupational Health fields and rural health.

1.6 Aims and Objectives of the PhD Project

This project had been carried out in the frame of a wide research project that is being conducted in the region of Lombardy, addressed at occupational exposure to biological risk factors among farmers and animal breeders. Therefore, the overall aim of this study has been collecting information regarding; 1. The exposure of workers to a selected group of biological agents (bacteria, viruses and organic dusts); 2. The identification of early immunological signs of effect; 3. The assessment of the immunity against tetanus of the agricultural workers of the region, considering different population subgroups. The project has been completed with the study of the respiratory function of these workers, carried out with a spirometer test.

Specific Objectives:

In this frame, the specific objectives of this project are:

To determine the seroprevalence of antibodies against HEV and other indicators of contact with biological agents (*Salmonella, Brucella, Coxiella, Leptospira, Borrelia*)

- To investigate the immunologic status (levels of immune system activation) to organic dust and other biological agents.
- To assess the level of immunity against tetanus among agricultural workers of the region

The general approach in the research was exposed (animal breeders) vs controls (non-breeders agricultural workers)

Chapter Summary

In this chapter agriculture, livestock, different types of risks for agricultural workers and in particular biological risks and important zoonoses in the study were discussed. On the other part respiratory disease in the agriculture were explained briefly. In the following chapter (chapter II) two studies will be explained in details; zoonoses study and immunity to tetanus.

CHAPTER II Zoonoses and Tetanus Immunity Study

2.0 Zoonoses Study

2.1 Aim of the Study

Since in Italy epidemiologic studies regarding occupational zoonotic risks are scarce, we conducted this explorative study in some rural areas of the Lombardy Region, which is well known for agricultural productivity, with the aim of defining, through the determination of specific IgG antibodies, the serologic evidence of contact with selected zoonotic agents *(Salmonella, Brucella, Coxiella, Leptospira, Borrelia),* among groups of agricultural workers engaged in different working activities at the workplace.
Overall, this study was aimed to evaluate the biological risks for the animal breeders, with a particular attention for HEV infection, considered as an emerging biological risk, and tetanus immunity.

2.1.1 Materials and Methods

Literature review

A systematic bibliographic search was conducted addressed at the following items:

1. publications pertaining to HEV infection or anti-HEV antibody prevalence in humans, or HEV on pig or swine or anti- HEV antibody prevalence in animals or the environment. The primary objective was to review and report on the current knowledge and evidence that exist on risk factors for hepatitis E and the most likely routes of HEV transmission. For this purpose, an electronic search of the United States National Library of Medicine and the National Institutes of Health Medical Database (Pubmed) from its inception was conducted in January 2010. Search words used were: (‘HEV’ or ‘hepatitis E’) and (‘zoonosis’ or ‘zoonotic’ or ‘epidemiology’ or ‘food’ or ‘environment’ or ‘animal’ or ‘pig’ or ‘meat’ or ‘farm’ or ‘farmer’). Finally among more than 1000 articles, 300 articles were related to HEV in human, animals, epidemiology of disease and laboratory gene sequencing methods.

2. Salmonella, *Brucella*, *Coxiella*, *Leptospira*, *Borrelia* Studies had to be relevant to the following topics: *Coxiella*, Q fever, tick-borne zoonoses, Lyme disease, *Leptospira*, and animal breeding and tetanus.

Our selection criteria for the bibliographic search was that articles and studies were included if they met the following criteria: peer-reviewed study or article; and reviews or books on relevant topics, such as tick-borne zoonoses or documents published by national and sovranational bodies, ministries, etc. Apart for the key words related to the specific biological risks mentioned, research has been addressed also at, biological risk, serologic studies in animal breeders, seroprevalence, epidemiology and farming.

Results of the literature review

Results for the literature review on the zoonoses related to the study are shown in **Table 2-1.**

Table 2-1. Results of literature review by categories of papers for zoonses.

Category	Full Text	Abstracts	Total
HEV in Animals	70	52	122
HEV in Human	62	30	92
HEV Epidemiology	10	12	22
HEV Gene Sequencing Tests	34	50	88
Pig and Zoonoses	18	32	50
Biogas in Enterprises	10	13	23
Slaughter house	2	2	4
Leptospira in Animals	10	20	30
Leptospira in Human	20	30	50
Leptospira Epidemiology	12	15	27
Leptospira in Italy	8	12	20
Coxiella in Animals	10	20	30
Coxiella in Human	19	30	49
Coxiella Epidemiology	15	40	55
Coxiella in Italy	4	15	19
Borrelia in Animals	15	40	55
Borrelia in Human	20	60	80
Borrelia Epidemiology	19	40	59
Tick Borne Diseases	11	10	22
Borrelia in Italy	5	40	49
Total	***374***	**563**	**946**

Study Settings

The research project consisted of two cross-sectional studies, conducted in 41 small size agricultural enterprises provided with occupational health surveillance at the workplace in the region of Lombardy by our Centre. The region is almost the heart of Northern Italy, bordered by Switzerland to the north, Piedmont to the west, Emilia Romagna to the south, Veneto and Trentino Alto Adige to the east.

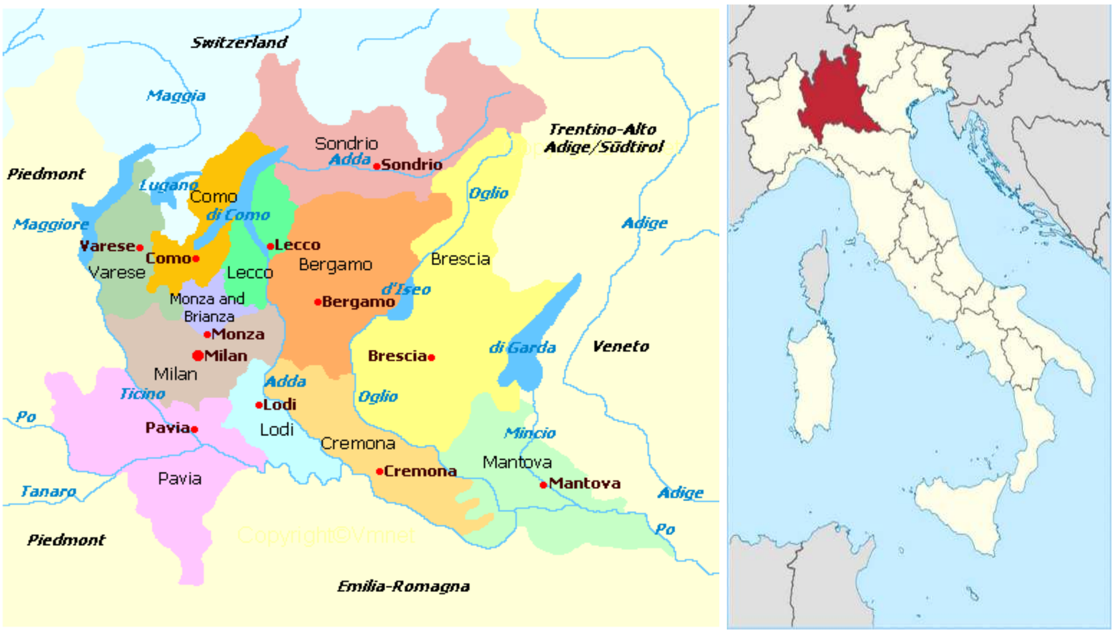

Figure 2-1. **Map of Lombardy Region, North of Italy**

Study participants

Our study participants were recruited from among 1000 workers who are being covered by occupational health care provided by ICRH (**Fig. 2.2**). These subjects are employed in about 400 enterprises in the region as shown in **Fig. 2.3.**

The two studies were conducted from January 2010 to January 2011 on two separate groups made of 199 randomly selected workers from 41 farms in the region, as described below. The first group under study (n=103, group A), was made of 47 cow breeders, 31 pig breeders, 11 fish breeders and 14 farmers that were not involved in animal breeding activities. The second group (n=96, group B), included 28 pig breeders, 36 cattle breeders, and 32 workers who were not engaged in animal breeding (control group). Eligibility for participation in the study was based on the following being healthy individuals, not older than 75, not having swine or cattle at home, have no liver disease and hepatitis history.

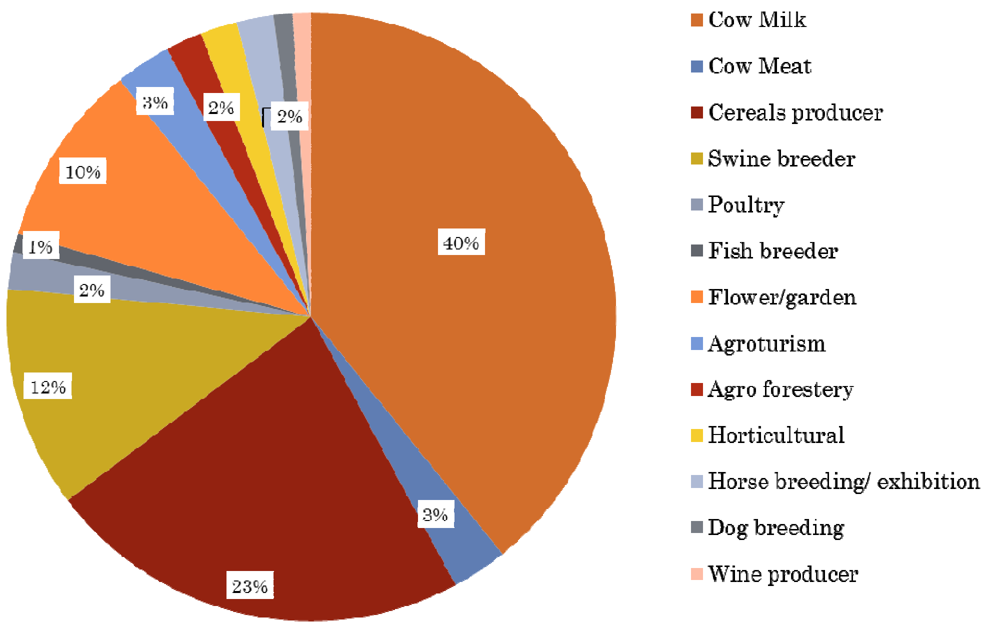

Fig. 2.2. The agricultural enterprises by their activities, covered by ICRH (n=400)

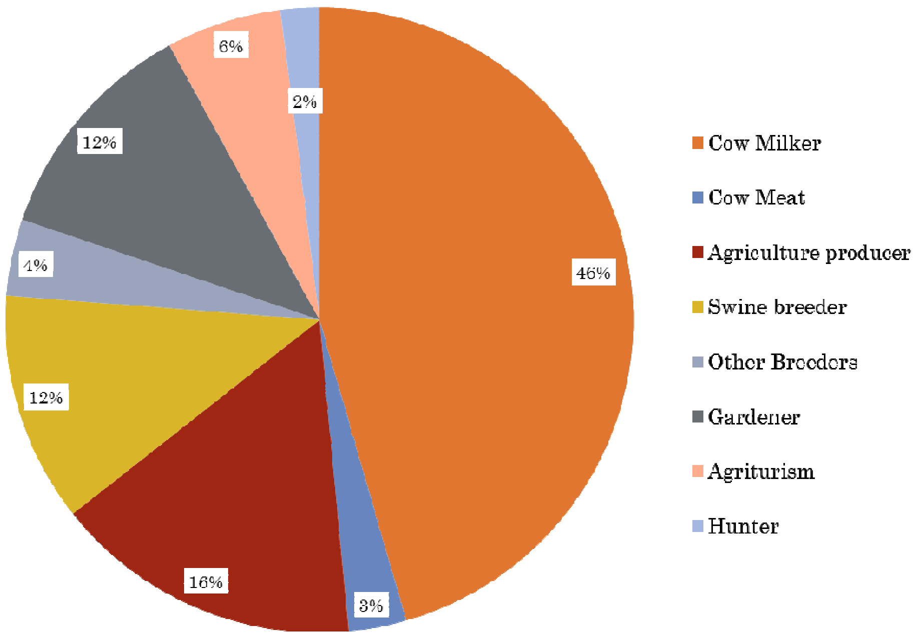

Fig. 2.3. Productive activities of the workers preovided with health surveillance at the workplace (n=1000)

Consent form

All workers were informed about the objectives and the methods of the study, approved by the ethical committee of the San Paolo Hospital (Comitato Etico, Ospedale "San Paolo"), and signed an informed consent for participation (**Appendix 1**).

Clinical Data and Personal Information Collection

Personal data such as socio-demographic and clinical information, personal habits, smoking and alcohol intake as well as the results of previous physical and laboratory examinations were recorded in the personal data collection form that is routinely used by our centre to collect data coming from individual health surveillance activities. Tobacco and alcohol consumption were assessed in a structured interview. Tobacco consumption was analysed with two variables, namely current daily smoking and the number of cigarettes smoked per day. Average alcohol intake, estimation of unit of alcohol consumption per day, type of beverage consumed.

Blood sample collection

Before physical examination, a 10 ml blood sample was obtained through venipuncture. Samples were processed within 4 hours of collection and isolated serum was frozen at −20°C until used.

Laboratory analysis

Group A (n=103)

In this group the presence of IgM and IgG antibodies against HEV was investigated through commercially available kits. Since recent published papers suggest different levels of sensitivity of commercially available kits for anti-HEV IgG detection, we tested workers' sera with two different kits (Assay 1: HEV Ab, Dia.Pro Diagnostic BioProbes, Italy –Assay 2: HEV IgG Elisa, Beijing Wantai Biological Pharmacy Enterprise, China) according to the manufacturer's instructions.

Group B (n=96)

In the subjects of the second group, the concentrations of antibodies against *Coxiella burnetii* and *Leptospira spp* were measured with a Complement Fixation Test (CFT)

(Diesse, Italy); the concentrations of antibodies against *Borrelia burgdorferi* were measured with Chemiluminescence method (Diasorin, Italy) and the concentrations of antibodies against *Brucella spp* and *Salmonella spp* were measured with a Direct Agglutination method (Diesse, Italy).

Statistical analysis

In the first group of 103 workers the prevalence of anti-HEV antibodies in the sera of workers was investigated. In the second group of 96 workers, the magnitude of the association between possible risk factors and seropositivity was expressed as odds ratios (ORs) with 95% confidence intervals (CIs), calculated with univariate and multiple logistic regression models to compare breeders and non breeders (reference group) adjusted for age, sex, country of origin (EU vs. non-EU), smoking (yes, no), and education (primary, secondary, high-school, higher level). Statistical analyses were carried out with the SPSS version 18 software (SPSS Inc., Chicago, IL, USA) . A value of $p < 0.05$ was considered to be significant.

2.1.2 Results

In the first group of 103 workers (102 male and 1 female), who were aged from 27 to 71 years (median 48) the majority of workers (74 out of 103, 71.8%) were Italian and 29 (28.2%) were from other nationalities (South Europe, Asia, Africa and South America). Socio demographic information related to the first group is shown in table 1.

Table 2-2. Demographic and personal information of the first study groups (breeders/ non breeders) n=103.

Job	**Age (years)** Median (Min-Max)	**Nationality** (EU-non EU)	**Smokers** (%)	**Cigarettes/day** Median (Min-Max)	**Alcohol Consumption** (%)	**Alcohol** units/day
Breeders (n=89)	48 (27-71)	74-15	24.71	20 (5-40)	39.32	2 (1-6)
Non breeders (n=14)	45 (30-75)	13-1	28.57	25 (20-30)	21.42	2 (1-3)
P value	0.76	0.09	0.35	0.07	0.9	0.85

Assay 1 revealed that among 103 samples from agricultural workers and animal breeders, none of the subjects showed the presence of IgM anti-hepatitis E, and only one breeder showed the presence of IgG anti-HEV (1%). Since the subject was an Indian cattle breeder, we assumed that very likely the contact with hepatitis E virus took place before migration to Italy. On the other hand, when the second commercially available kit was used, results were completely different from those obtained with the first kit and the prevalence of antibody titres raised from 1 to 22.3%.

Table 2-3 compares the results of two commercial anti HEV IgG assays on study subjects.

Table 2-3 Comparison of two type of assays used in the study (n=103)

JOB	**Assay I**		**Assay 2**	
	+	**%**	**+**	**%**
Breeder	1/90	1	23/90	25.6
Non Breeder	0/13	-	0/13	-
Total	*1/103*	***1***	*23/103*	***22.3***

The second study was conducted on a group of 96 workers (91 males, 5 females), aged between 19 to 70 (Median 42 years). Socio-demographic information related to the second group is shown in **Table 2-4.**

Table 2-4. Demographic and personal information of the second study group n=96.

Workers/Variables	**Age (years)** Median (Min-Max)	**Nationality** (EU-non EU)	**Smokers** (%)	**Cigarettes/day** Median (Min-Max)	**Alcohol consumption** (%)	**Alcohol** units/day
Breeders (n=64)	44 (19-70)	42-22	21.9	17.5 (2-30)	40.6	2 (1-12)
Non breeders (n=32)	34 (22-62)	28-4	34.4	10 (5-25)	65.6	1.5 (1-5)
P value	0.00	0.02	0.22	0.63	0.02	0.21

The results for the prevalence odds ratios and 95% confidence intervals of seropositivity to antibodies against selected zoonotic agents in agricultural workers are shown in **Table 2-5,** Results of logistic regression analysis, adjusted for age, sex, country of origin, smoking , and education revealed that breeder workers show a tendency to have higher prevalence of positivity for antibodies to *Leptospira* and *Coxiella* than farmers, more evident in the multiple logistic regression analyses (ORs around 3). We found little difference for *Borrelia* in the crude analysis, and we did not perform a multiple regression analysis because the small number of positives.

Table 2-5. Prevalence Odds ratios (OR) and 95% confidence intervals (CI) of positivity to antibodies against selected zoonotic agents by job title. Results of univariate and multiple logistic regression models.

Zoonotic agent	No. Positive	%	OR Crude	95% CI	OR Adjusted*	95% CI
Leptospira						
Farmers	14	43.7	1.00	(Reference)	1.00	(Reference)
Breeders	38	**59.4**	**1.46**	0.62,3.46	**3.03**	1.01,9.15
Swine-breeders	*15*	*53.1*	*1.23*	*0.44,3.43*	*2.40*	*0.71,8.17*
Cattle-breeders	*22*	*61.1*	*1.68*	*0.63,4.43*	*4.02*	*1.10,14.77*
Coxiella						
Farmers	10	28.1	1.00	(Reference)	1.00	(Reference)
Breeders	32	**51.6**	**2.10**	0.86,5.16	**2.97**	0.92,9.58
Swine-breeders	*14*	*50*	*2.10*	*0.73,6.04*	*2.91*	*0.81,10.45*
Cattle-breeders	*18*	*50*	*2.10*	*0.77,5.69*	*3.05*	*0.82,11.37*
Borrelia						
Farmers	4	12.5	1.00	(Reference)	1.00	(Reference)
Breeders	5	7.8	0.59	0.15,2.38	NE	
Swine-breeders	*4*	*14.3*	*1.17*	*0.26,5.17*	*NE*	
Cattle-breeders	*1*	*2.8*	*0.20*	*0.02,1.89*	*NE*	

*Adjusted for age, sex, country of origin (EU vs. non-EU), smoking (yes, no), and education (primary, secondary, high-school, higher level). NE, not estimated.

Serum protein electrophoresis results between breeders and farmers and between cattle and swine breeders are shown in **Table 2-6**. Our results indicate slightly higher values of serum proteins and globulins in animal breeders compared to control group. Furthermore, there was a statistically significant difference between occupation (animal breeders vs. farmers) on albumin and alpha-1 globulin. Slightly higher values of total serum proteins were observed in breeders than in farmers. All the results were in the range of normality for all the subjects involved in the study and altogether these findings do not shown any biologically relevant difference between groups for the tested variables.

Table 2-6. Serum protein indices by job title. Results of multiple linear regression models (n=96).

Serum proteins	No.	Mean	Adjusted difference*	95% CI
Albumin				
Farmer	30	4.56	(Reference)	-
Breeder	60	4.64	0.16	0.01, 0.32
Swine	*26*	*4.80*	*0.28*	*0.11, 0.45*
Cattle	*34*	*4.52*	*0.04*	*-0.12, 0.21*
α1				
Farmer	30	0.14	(Reference)	-
Breeder	60	0.15	0.01	-0.00, 0.02
Swine	*26*	*0.14*	*0.00*	*-0.01, 0.02*
Cattle	*34*	*0.16*	*0.02*	*0.00, 0.04*
α2				
Farmer	30	0.69	(Reference)	-
Breeder	60	0.71	0.02	-0.02, 0.06
Swine	*26*	*0.70*	*0.02*	*-0.03, 0.07*
Cattle	*34*	*0.75*	*0.03*	*-0.02, 0.07*
β				
Farmer	30	0.88	(Reference)	-
Breeder	60	*0.93*	0.03	-0.03, 0.08
Swine	*26*	*0.93*	*0.03*	*-0.03, 0.09*
Cattle	*34*	*0.93*	*0.02*	*-0.04, 0.09*
γ				
Farmer	30	*1.08*	(Reference)	-
Breeder	60	*1.12*	*0.00*	*-0.13, 0.13*
Swine	*26*	*1.12*	*0.02*	*-0.12, 0.17*
Cattle	*34*	*1.10*	*-0.02*	*-0.17, 0.13*
Total serum protein				
Farmer	30	*7.36*	(Reference)	-
Breeder	60	*7.45*	*0.16*	*-0.24, 0.56*
Swine	*26*	*7.69*	*0.36*	*-0.88, 0.82*
Cattle	*34*	*7.25*	*-0.47*	*-0.50, 0.41*

*Adjusted for age, country of origin (EU vs non-EU), smoking (yes, no; and cigarettes/day), and BMI.

2.1.3 Discussion

The aim of the first study was investigating seroprevalence of antibodies against hepatitis E virus (HEV) in different animal breeders and non exposed group (farmers) . Serological studies regarding HEV prevalence in Italy are scarce and little is known about occupational exposure in farmers and animal breeding enterprises. Studies in Western Europe and United States indicate that presence of antibody to HEV (anti-HEV) is more common than expected in areas where HEV infection is not endemic and the cases of Hepatitis E are rarely reported. HEV-related viruses have been found in pigs, wild boar and deer as well as in rodents and chickens. Direct transmission has been reported from animals to humans through consumption of undercooked deer meat or uncooked liver from a wild boar. Evidences suggest that those who consume contaminated pork products or are involved in the pigs breeding activities are potentially at risk of HEV infection (Meng, 2010).

Some studies suggest that HEV infection may be an asymptomatic zoonotic infection in industrialized countries (Teo 2006; Drobeniuc *et al*.; Pavio *et al*., 2010). Galiana *et al* (2008) carried out a study to investigate the prevalence of HEV and the risk factors for the acquisition of the virus in a population in contact with swine and unexposed to swine in Spain. Their results indicated that swine breeders suffered a 5.4 times higher risk of having anti-HEV IgG compared to control group (P <0.03). In this light, HEV infection should be seriously considered as a possible occupational disease in these workers. As for Italy, the prevalence of anti-HEV in the general population is estimated around l-5 % healthy Italian population (La Rosa *et al*, 2011; Romanò *et al*., 2010; Aggarwal *et al*, 2011; Coppola *et al*., 1998).

In our study the prevalence of anti HEV in agricultural workers using assay 1, was 1%, which is similar to the seroprevalences reported in literature, that also suggest no significant difference for anti-HEV seroprevalence between swine farmers and general population (2.9% and 3.3% respectively) in the Latium region (Vulcano *et al*., 2007). Enzyme immunoassays based on recombinant proteins of HEV have been used for most seroprevalence studies. A wide range of sensitivity and specificity has been reported for these assays. It is important to underline that the sensitivity and specificity of the assays used to test for IgG and IgM anti-HEV have not been well established in areas where hepatitis E is not endemic (Chen Chun *et al.*, 2000).

The present study compared the sensitivity of two commercially available kits HEV IgG EIA and the results suggest that kit 2 is more sensitive than kit 1 (22.3% and 1%, **Table 2-3**). There are several commercial serological assays for the detection of anti-HEV IgG (Panda *et al*., 2007) for instance; the Genelabs EIA has been the most commonly used worldwide. Several studies implied that Wantai assay (assay 2) has higher seropositivity than the Genelabs assay, which may suggest the higher sensitivity of the Wantai assay (Park *et al.*, 2012). Bendall *et al.*(2010) compared the performance of Genelabs and Wantai HEV IgG EIA kit using World Health Organization standard sera; the Wantai assay was more sensitive than the Genelabs assay, and continued to test infected individuals as positive for longer periods post-infection. The authors also tested 500 blood samples obtained from blood donors in the United Kingdom using both assays; the Wantai assay resulted in a substantially higher estimate of seroprevalence (16.2%) than that of Genelabs (3.6%). It is noteworthy to mention that Mansuay *et al.*, (2011) by using a validated sensitive assay (Wantai) found hepatitis E virus IgG in 52.5% of voluntary blood donors in southwestern France.

With regards to the above studies and considering the fact that assessing the specificity of the tests is difficult in situations other than acute hepatitis E (lack of gold standard for checking the specificity of the current anti-HEV ELISA kits), further studies are required to clarify the epidemiology and risk factors for HEV infection in animal breeding and agricultural workers.

In conclusion, the differences in seroprevalence rates between different populations must be interpreted with caution (Mushahwar, 2008), it might be due to the fact that demographic variables, such as age, are related to the prevalence, and also because the assays vary in their sensitivity (Purcell and Emerson, 2008; Park *et al.*, 2012).

In the second pilot study we investigated other important zoonotic risks among agricultural workers, including Leptospirosis. *Leptospirosis* is one of the zoonotic infections which is widely seen especially in tropical regions, and the primary transmission is by direct contact of water, contaminated by the secretions of animals. Leptospirosis is endemic in many countries, perhaps in the whole world. It often has a seasonal distribution, increasing with increased rainfall or higher temperature (WHO website). Leptospirosis is of increasing importance as an occupational disease as intensive farming practices become more widely adopted. During 1999, those working in agricultural industries in Australia accounted for 35.3% of notifications while those working in livestock industries accounted for 22.9% of notifications (Smythe *et al*., 2000).

In our study there was a relatively high prevalence of IgG to *Leptospira spp* among workers (54.1%). This percentage is higher than reported in previous studies, suggesting a range between 5.6 and 40 % (Cerri *et al*., 2003; Crevatin *et al*., 1986). Moreover, our study did not point out any significant difference between breeders and non-breeders and among different groups of breeders. Leptospirosis is still a public and occupational health problem in Italy: in a three year period (1994–1996), 222 reports of human cases of Leptospirosis from 16 regions of Italy were received by the Italian Ministry of Health and results implied that 18.2% of these cases, were by direct contact with other animals and specially swine (Ciceroni *et al*., 2000). In Italy, leptospirosis is a disease reported both in animals and humans. In fact, a study conducted in northern and central Italy from 1995–2001 demonstrated that 6.81% of animals and 5.6% of humans sera scored positive to the presence of antibodies to *Leptospira* (Cerri *et al*., 2003).

Until the 1960s, leptospirosis was a moderately common occupational infection, especially in rice field workers, due to prevalence of wild rats and farmers and animal breeders can be infected by direct or indirect contact with the urine of infected animals (Sambasiva *et al*., 2003). Mono *et al.* (2009) carried out a survey in south of Italy to investigate the seroprevalence of zoonoses in two cohorts of farm workers and blood donors (Monno *et al*., 2009). None of the subjects had antibodies against *Brucella* and *Leptospira*. Serologic studies on animals have shown different results for *Leptospira,* from 18% in wild boars in Germany, to 20-53% in rodents in France (Jansen *et al*., 2007; Aviat *et al*., 2009). According to WHO report, incidences range from approximately 0.1–1 per 100 000 per year in temperate climates to 10–100 per 100 000 in the humid tropics. During outbreaks and in high-exposure risk groups, disease incidence may reach over 100 per 100000 (Massarani, 2004). Overall, our study suggests that the contact with *Leptospira* is very common in agricultural workers, and that the risk is not related with breeding activities but only with the condition of “agricultural worker”, being this condition alone able to bring about an increased possibility of contact with surface water, possibly contaminated by rodents’ urine.In conclusion,our data suggest that *leptospira* is still a risk for agricultural workers and should be considered in preventive activities and health surveillance programmes.

In the current study, one of the zoonotic agents with higher prevalence in the animal breeders comparing to farmers was *Coxiella burnetti*. According to literature, persons at risk for Q fever include abattoir workers, veterinarians, farmers, and other individuals who have contact with infected animals, particularly newborn animals, or products of conception like placenta. In EU region, there was an outbreak of Q fever in the Netherlands in 2007, and by August 2009 more than 2000 cases had been reported, with 11 fatalities (Walker *et al*., 2012). Despite the widespread prevalence of infection with *Coxiella burnetii*, there have been few large population-based surveys that investigated the epidemiology of this infection (McCaughey *et al*., 2008).

Thomas *et al* (1995) conducted a survey in the United Kingdom to investigate seroprevalence of *Coxiella bornetti* in 2 cohorts of farmers and control groups (police and emergency service personnel). Results revealed that 105 out of 385 farm workers were found seropositive to *C.burnetti* and no association between seoprevalence and age was found. They concluded that risk of having antibodies to *Coxiella* burnetii increases with exposure to a farm environment. Therefore, full time farmers were more likely to have acquired antibodies than part time farmers, and prevalence was higher in regular hired workers (Thomas *et al*., 1995). It is intmportant to remember that an efefctive vaccine against Q fver is available in Italy and in the whole european Union: the results of our study might suggest the need of considering specific workers' subgroups for vaccination. Italy has a mandatory notification system for all rickettsial infections including Q fever.

Manfredi Selvaggi et al (1996) reported an outbreak of 58 cases during summer and autumn 1993 in the Veneto region. The case control study showed a significant association with exposure to flocks of sheep. In Germany, two outbreaks of Q fever were investigated; in 1992 one occurred in a Berlin research facility where sheep were kept and the other in 1993, in a rural area in Hessen. In both outbreaks infected sheep were suspected to be the source of the outbreaks (Eurosurveillance, 1997). Santoro *et al* (2004) reported an outbreak of Q fever in Como, north of Italy in 2003. One hundred and thirty three cases of acute Q fever with clinical symptoms (high fever, dry cough, arythromyalgia, fatigue and chest abnormalities) with confirming serologic results, were reported to department of prevention of the local health unit. Here infected sheep were also suspected to be the source of the outbreaks.

Our study results showed that 42 workers out of 96 (43.7%), mainly breeders workers had anti *Coxiella* IgG in their serums, so more contact with animals has resulted in increasing antibodies in their peripheral blood. One of our study limitations was that we did not have the chance to investigate about the prevalence of zoonoses in sheep and goat breeders in the region.

Lyme diseease (L.D.) is now the most common vector-borne illness in the United States and Europe. Since surveillance was begun by the Centres for Disease Control and Prevention (CDC) in 1982, the number of cases in the United States has increased dramatically. Around 25,000 new cases are reported each summer and in Europe, the highest reported frequencies of the disease are in the middle of the continent and from Scandinavia (Steere, 2008; CDC website). There have been some seroprevalence studies on humans in the Europe. The highest incidences of LB in Europe are found in the Baltic States and Sweden in the north, and in Austria, the Czech Republic, Germany, Slovenia and central Europe (Pascucci et *al.*, 2010).

Chiemlewska-Badora (1998) investigated anti Borrelia burgdorferi anitbodies in groups of foresty workers, farmers and blood donors in the Lublin region of Poland . The results showed highest rate of seropositivity belonged to foresty workers and farmers (38.6% and 28.1% respectively) than blood donors as a control group (6%). Lyme disease is also the most widespread tickborne disease in Italy. Risk areas for Lyme disease are limited primarily to northern Italy along the Ligurian coast, and the Adriatic coast (WHO 2007). Our study results pointed out that 9.4% of the workers in the study had anti *Borrelia* IgG in their serums which is higher than results obtained by Di Renzi *et al.,* (2010) in forestry workers (3.4%) and below the values of Chiemlewska-Badora (2008) study reslts in Lublin, eastern Poland (13-33% in exposed groups) (Di Renzi *et al*, 2010; CisakE *et al*., 2008). The other point is, our laboratory results impled less anti-Borrelia in cattle- breeders workers comparing to pig breeders and non breeders (2.8%, 14.3% and 12.5%).

Several studies and clinical observations show that serum protein concentrations may be altered as a result of different disease states (e.g hepatic diseases) and interpretation of serum protein electrophoretic patterns can be helpful in confirming the diagnosis of some diseases (Seibert *et al*., 1947, Foley *et al.*, 1937). Our results showed a very small but statistically significant difference between animal breeders and non breeders serum concentration of albumin and alpha-1 globulin. This might be due to increased exposure to biological agents in animal breeders, especially in swine breeders (e.g. acute/chronic infections etc).

In general, the results of our study, also through statistic elaborations including univariate and multivariate regression analysis, indicate that animal breeders had higher percentage of antibodies to *Coxiella* and *Leptospira* comparing to other farmers who were not engaged in animal breeding activities.

2.2 Immunity to Tetanus study

2.2.0 Introduction and Aim

Despite the fact that tetanusvaccination is compulsory for agricultural workers in Italy and occupational health physicians should check workers' immunisation status during their periodic workers’ health surveillance, the information about serum levels of tetanus antitoxins in farmers and specially immigrant workers is still scant. For this reason, we decided to integrate our study with an investigation on the levels and the prevalence of tetanus immunity in the study groups.

2.2.1 Materials and Methods

This cross-sectional study was conducted in the same group of small size agricultural enterprises covered with occupational health surveillance at the workplace in the region of Lombardy described in the previous study.

In the first phase of the study, one hundred and fifty one farmers and animal breeders were involved. All the participants were asked wether they remebered to be vaccinated against tetanus and also about the vaccination time.

After the first phase, all the workers were informed about the aim of the study and signed an informed consent for participation. Personal data such as socio-demographic, clinical information and the results of previous physical and laboratory examination were recorded in the personal data collection form that is routinely used by our centre to collect data coming from individual health surveillance activities. Concentrations of antibody to tetanus toxin (antiTT) in sera were measured by an enzyme-linked immunosorbent assay. Anti TT levels of ≥ 0.11 IU/mL were considered protective (**Table 2.7**).

Table 2-7. Levels of immunity (ref values) for tetanus protection

IU/ml	Tetanus protection
<0.01	No protection
0.01–0.1	Dubious
>0.1–0.5	Short term immunological protection
>0.5–1	Immune protection (3 years)
1–5	Long term protection (~ 5 years)
5–10	Long term immunity (~ 8 years)

(Maple *et al*., 1995; Plotkin 2001; Weckx *et al*., 2006; FDA website)

Before physical examination, a 5 ml blood sample was obtained through venipuncture. Samples were processed within 4 hours of collection and isolated serum was frozen at −20°C until used.

SPSS 18 for Windows® was used for statistical analysis of the data. Mean, standard deviation and percentages were used in evaluation of descriptive statistics. The levels and the prevalence of tetanus immunity was studied in various workers subgroups, in particular considering age and country of origin. The chi-square test was used for qualitative data. Linear regression was used for correlation analysis. A value of $p < 0.05$ was considered to be significant. The study was approved by the ethical committee of San Paolo hospital of Milan (Comitato Etico, Ospedale "San Paolo").

2.2.2 Results

One of the main questions in the study was whether the workers remembered they have been vaccinated against tetanus or not. In our survey nearly half of the workers declared that they had vaccination (48.6%) while 38.6 % gave negative answer and the rest had no data on that. In general, European workers had more positive response than non-EU workers (53.2% and 27.9% respectively), as it is indicated in **Figure 2.4.**

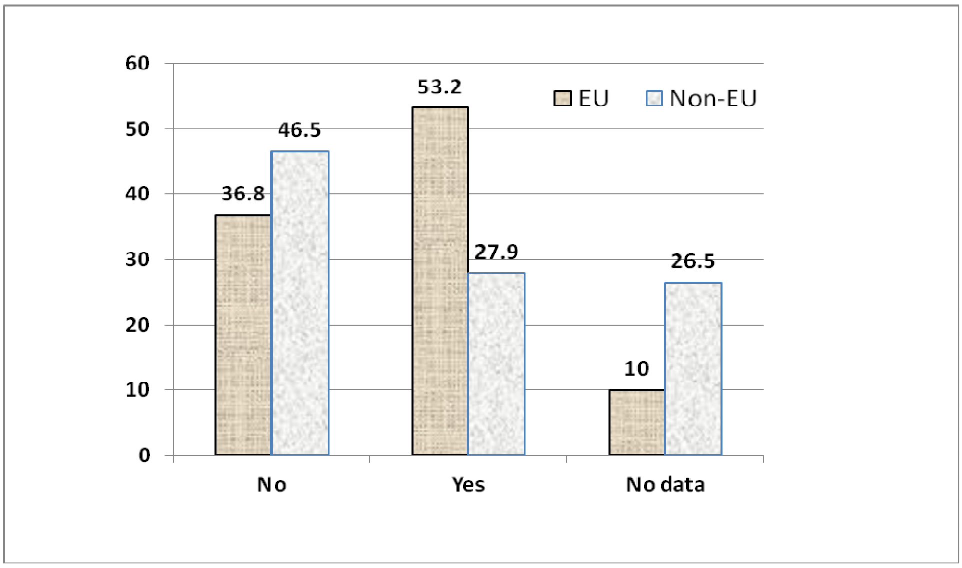

Figure 2.4. Answer to the question"do you remember to be vaccinated against tetanus?" in EU and Non-EU workers.

Figure 2.5 shows the results in 3 groups of workers by their nationality. It is shown that Italian workers had higher probability of positive answer compared to the workers from East European countries or non-EU workers (55.1%, 25% and 27.9%, respectively).

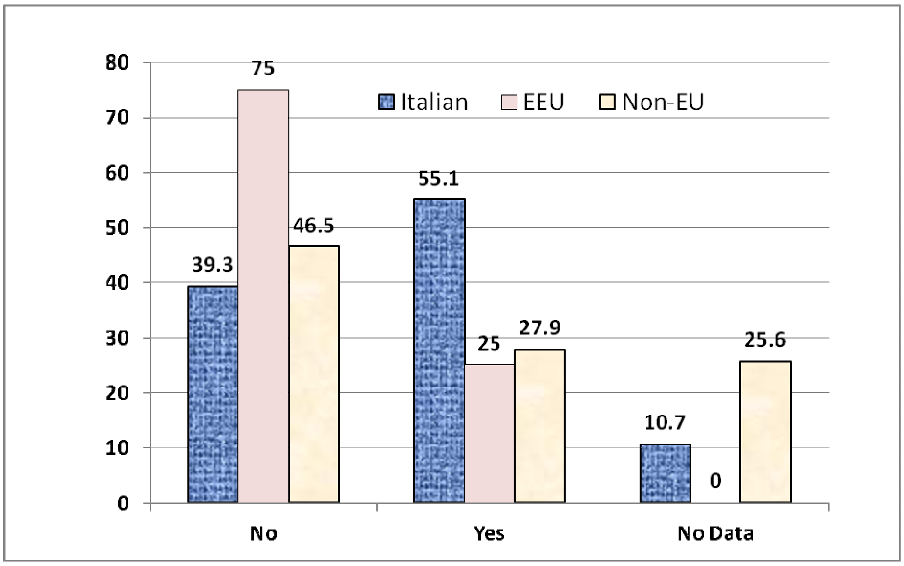

Figure 2.5. Answer to the question"do you remember to be vaccinated against tetanus?" in IT/ EU and non-EU workers.

Among 151 workers (151 male and 6 female), who were aged from 21 to 72 years (mean 44) the majority of workers (99 out of 151, 66%) were Italian and the rest (34%) were from other nationalities (South Europe, Asia, Africa and South America). Socio demographic information related to the workers is shown in table 2-7.

Table 2-8. Demographic characteristics for the study groups by their job (n=151).

Workers/ Variables	**Age (years)**	**Nationality**		**Age groups**				
	Med. (Min-Max)	EU	NEU	<19	20-29	30-39	40-49	+50
Breeders (n=93)	45.6 (21-72)	60	33	3	6	18	28	33
Non breeder (n=58)	41.3 (24-72)	39	19	-	13	18	15	11
P value	**0.63**	**0.73**		**0.006**				

Keys: EU, European; NEU, Non European; Med, median; Min, minimum; Max, maximum

Figure 2-6 represents immunity to tetanus in agricultural workers (n=151). As the figure illustrates, Italian workers show better immunity to tetanus compared to non-European workers: In fact, tetanus immunity long term protection (> 1 IU/ml) for European and non-European workers were 60% and 41.7% respectively and this difference was statistically significant (P <0.01, chi square test). There were no worker with no protection (<0.01) and 7.8% of EU and 25% of non-EU workers had protection at dubious level. Immunization of workers against tetanus was investigated through detection of anti-tetanus IgG.

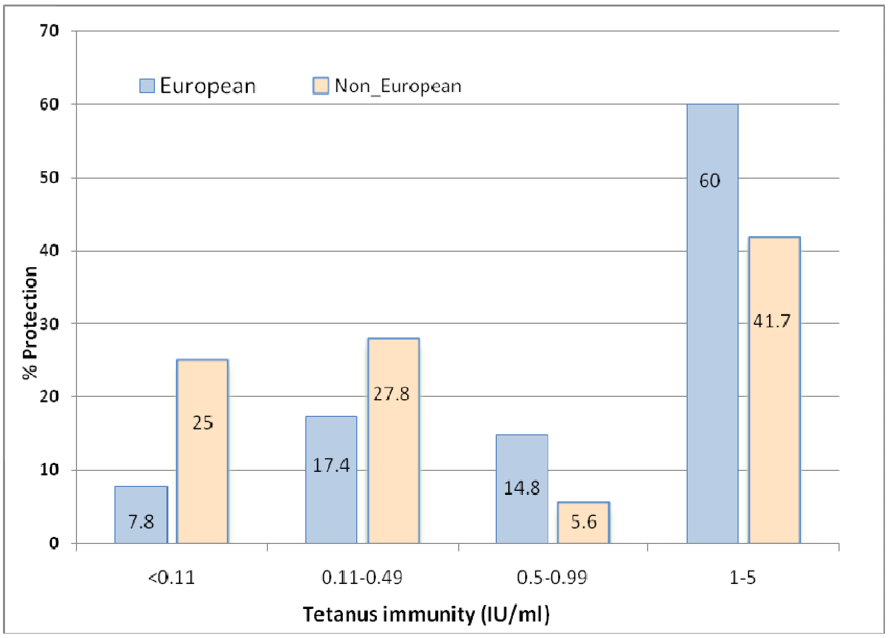

Figure 2-6. Comparing immunity to Tetanus in agricultural workers by their nationality (n=151).

Figure 2-7 illustrates the percentage of the workers that showed a condition of immunization to tetanus. It was shown that Italian workers, compared to non-European workers, had better immunity to Tetanus. The figure also suggests that in both groups there was a decrease in immunity with increasing of age (over fifty years of age).

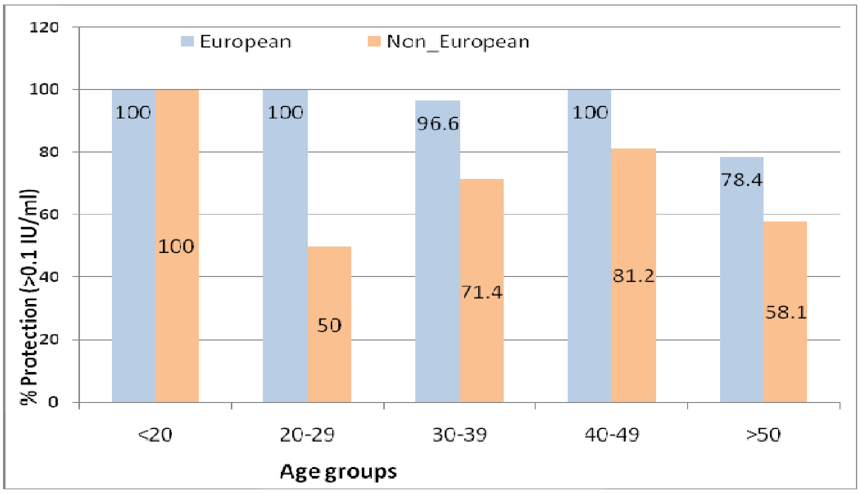

Figure 2-7. Distribution of agricultural workers protected against tetanus by age groups and nationality.

Results of the multivariate linear regression showed among independent variables (gender, type of job, nationality and BMI) nationality and age were significant predictors (P<0.01) regarding to dependent variable (anti TT titer).

2.2.3 Discussion

Despite widespread immunisation of population in Italy, tetanus still occurs in this country. In Italy, tetanus vaccination became mandatory for military recruits in 1938. In 1963, recommendations were extended to include children during the second year of life and selected occupational groups considered to be at elevated risk (e.g., those working in agriculture and animal husbandry). Tetanus reporting has been mandatory in Italy since 1995. It is considered a class I condition, that requires notification within 12 hours of a suspected case. Among the 292 cases reported to the Istituto Superiore di Sanità in 1998-2000, 181 (62%) had information available on vaccination status (56% of men and 65% women). The decline in tetanus cases has been paralleled by a decline in case-fatality, from 64% in the 1970s to 40% in the 1990s (Epicentro, 2000). In our study, among 151 workers , 126 workers had immunity to tetanus (*anti TT ≥0.11 IU/ml)* and Italian/EU workers had better immunity than non EU workers (93% vs. 77.8%) and this difference was statistically significant (p<0.01, Chi-square test).

A study of the immunization status in industrialized countries shows that elderly citizens and agricultural workers usually have very low protection against tetanus that might be due to poor level of delivery of health care in rural areas (Pascucci and Rapisarda 2001). Nowadays, mandatory vaccination programme at younger ages, has shifted tetanus to older adults who are inadequately vaccinated. Since 1930, most tetanus cases in the United Kingdom have occurred in older people, mainly women, who had never been vaccinated (Galbraith *et al.*, 1981; Eurosurveillance, 2002). Evidence suggests that between two and seven tetanus cases per year occur in England and Wales (Eurosurveillance, 2002).

In the present study, age was found as a significant variable affecting the immune status of the participants. In our study, it was found that in subjects aged more than fifty, 78.4% of Italian-EEU workers and 58.1% of non-EU workers had protective levels against tetanus. The protective levels of immunity were more prominent at younger ages, as it was shown in our workers; youngers (with an exception for non-EU workers) generally had better immunity than older workers. As tetanus vaccination is not mandatory for late adulthood and elderly people, it may not be surprising to find low levels of immunity which is declining with increasing age. Frequency of injuries, possible lack of vaccination history, and decrease of general as well as specific immunity in elderly people make them vulnerable group for tetanus infection (Janout et al., 2005). In some developing countries, it was reported that many elderly people especially in rural parts of the country are unvaccinated or have lower level of immunity against tetanus (Razzaghi *et al.,* 2011; Oztürk *et al.,* 2003).

Different studies have shown that rate of protection against tetanus varied according to the study population. According to del Corro, *et al.*, (2009). In Spain, tetanus has been a statutorily notifiable disease since 1982 and adult immunization against tetanus seems inadequate. They recommended increasing health promotion education programmes as a strategy to reach the correct tetanus vaccination coverage. Seroepidemiological surveys conducted in western countries have shown that a considerable percentage of the adult population does not display protective levels of circulating tetanus antibody (del Corro *et al.*, 2009).

According to evidence there was a marked decrease in mortality from tetanus from the early 1900s to the late 1940s in US. In the late 1940s, tetanus toxoid was introduced into routine childhood immunization and tetanus became nationally notifiable. At that time, 500–600 cases (approximately 0.4 cases per 100,000 population) were reported per year. However, during 2001 through 2008, a total of 233 tetanus cases was reported an average of 29 cases per year (CDC website).

In our study females were a small fraction of the selected workers (6/151, 4%). Overall our results implied that among workers, 11.7 % of males and 16.7% of females did not have enough protection against tetanus and for long term immunity the ratio was 55.9% and 50% for males and females respectively. It was shown through different studies that male farmers are facing more injuries at their work places and it was reported that male workers had better immune status comparing to female workers (Oncu, 2011), however in some studies indicating report seroprevalence as being higher in males (Padelino 2002, del Corro 2011) in our study there were no significant difference between the sexes. Our results also suggest that compliance with vaccination program is decreased after the fifth decade of life.

With regards to the **Fig 2.4** results, it was shown that nearly half of the workers (46%) did not have the booster dose recently for tetanus, do not have a document or do not remember to be vaccinated. We believe that our studies would support the recommendations of booster immunisations for agricultural workers, especially migrant workers and elderly individuals who might not have previously completed the immune vaccination against tetanus. It is important to underline that, even if the worker does not remember to be vaccinated, he/she is very likely immunized as evident in some cases showing very low immunization levels but infact we had no worker with zero immunity to tetanus.

Finally, preventing tetanus should be a high priority for all primary care physicians and health personnel. Active immunisation with tetanus toxoid is extremely safe and effective, having in mind that the personal history collection of past immunisation can be not the best predictor of current immune status of agricultural workers, in particular for immigrants and elderly. It is important that physicians and occupational health staff know that the gold standard in the decision making process is represented by antibody titer determination but also that when this cannot be done, the performance of a booster doses the most appropriate solution, able to ensure an adequate coverage of most of the workers addressed.

Chapter Summary

In this chapter two type of studies on the agricultural workers has been explained; in the first study, zoonoses, 199 (103 + 96) workers were participated. In the second study, immunity to tetanus, 151 workers were tested for the level of immunity to this vaccine preventable disease. In the following chapter (chapter III) we will explain about workers' immune system response to the biological agents and organic dusts.

CHAPTER III Immune System Activation and Spirometry

3.0 Aim

This research was conducted with the aim of studying selected immune parameters in a group of animal breeders (exposed) and in agricultural workers not engaged in animal breeding (controls) to evaluate the presence of possible differences between the groups, suggesting of the possible presence of job related patterns of activation/ deactivation of immune system.

3.1.1 Materials and Methods

Study Subjects

A total of ninety six subjects were selected (group A= 91 males and 5 females), 64 animal breeders (exposed group) and 32 rural workers engaged only in agricultural activities but not in animal breeding (control group). The exposed group included 36 cattle and 28 pig breeders. Subjects were selected according to the guidelines of the Italian Health authorities and to the Declaration of Helsinki principles. Criteria for exclusion were intake of medications known to affect the immune system, i.e. steroids and non-steroidal anti-inflammatory drugs, recent vaccinations, or presence of malignancies, inflammations, infections as well as conditions of immunodeficiency. None of the workers showed any of these conditions when we conducted the study.

Clinical Data and Personal Information Collection

Personal data such as socio-demographic and clinical information, personal habits, smoking and alcohol intake as well as the results of previous physical and laboratory examination were obtained from the personal data collection forms routinely used by our Centre to collect health surveillance individual data sheet.

Blood Sample Collection

Before physical examination, 10 ml blood samples were obtained through venepuncture. Within 4 hours after collection, blood samples were processed and isolated serum was frozen at −20°C until analysis. Samples were collected in the morning, before the beginning of the work shift.

Blood analysis. The following parameters were measured: complete and differential blood cells count, serum proteins, including α1, α2, β and γ-globulins and total serum proteins, and cytokines (IL-6, IL-8, IL-10, TNF-α, IFN-γ). Cytokine production was assessed by ELISA using commercially available kits (Immunotools, Friesoythe, Germany) following the manufacturer's instructions. Results are expressed in pg/ml. The limit of detection was 1 pg/ml for all cytokine assessed.

Blood cell count was measured with an automated hematology analyzer: the Sysmex XT-2000*i* (Dasit, Milan, Italy). Calibration of the instrument was confirmed each day using two levels of controls (Sysmex e-Check Xe Hematology Controls for Sysmex x-series Analyzers, Sysmex Italy), according to the manufacturer's recommendations. Repeated analysis of a sample obtained from a healthy donor was used daily to confirm instrument precision. The between-run imprecision (CV%) was < 6.97% for all the parameters.

Serum proteins were measured by semi-automated agarose electrophoresis with the Hydrasys LC automate (Sebia, Florence, Italy). In the staining compartment, staining (4 minutes with amido black), destaining (three times, for 3 minutes, 2 minutes and 1 minutes, respectively), and drying (75°C for 8 minutes) were done automatically and finally the gels were scanned with the Hyrys densitometer (Sebia, Florence, Italy). Each day, two assays with two aliquots of a serum pool were analysed. The serum pool was prepared from patient specimens filtered through 8 µm (pore size) filters. The pool was aliquoted and stored at –20° C until assay. The between-run imprecisions (CV%) were 1.50%, 5.61%, 2.95%, 2.46% and 2.8% for the albumin, α1-globulin, α2-globulin, β-globulin and γ-globulin fractions, respectively.

3.1.2 RESULTS

Ninety one males and 5 females of age between 19–70 years (Median: 42 years) were enrolled in the study. They included 64 breeders (28 pig breeders and 36 cattle breeders) and 32 non-breeders. All workers were in good health conditions, without any sign of health impairment. Data regarding demographic and personal information are shown in **Tables 3–1** and **3–2**. **Table 1** compares data between breeders and non breeder workers in the selected enterprises while **Table 3–2** represents data only for breeders (swine and cattle). There was a significant difference for alcohol intake (p=0.02, Mann-Whitney test) between the study groups (breeders/ non breeders) while no significant difference was detected for weight and smoking habits of the Participants (see **Table 3–1** for details).

Table 3-1. Demographic and personal information of the study groups (breeders/non breeders)

Workers/Variables	Age (years) Median (Min-Max)	Weight(kg) Median (Min-Max)	Nationality (EU-non EU)	Smokers (%)	Cigarettes/day Median (Min-Max)	Alcohol consumption (%)	Alcohol units/day
Breeders (n=64)	44 (19-70)	75 (60-105)	42-22	21.9	17.5 (2-30)	40.6	2 (1-12)
Non breeders (n=32)	34 (22-62)	76 (52-100)	28-4	34.4	10 (5-25)	65.6	1.5 (1-5)
P value	0.00	0.63	0.02	0.22	0.63	0.02	0.21

Table 3-2. Demographic and personal information of the breeders (swine/cattle breeders)

Workers/Variables	Age (years) Median (Min-Max)	Weight(kg) Median (Min-Max)	Nationality (Eu-non EU)	Smokers (%)	Cigarettes/day Median (Min-Max)	Alcohol consumption (%)	Alcohol units/day
Swine breeders (n=28)	39 (19-70)	74.5 (60-105)	22-6	25	17.5 (2-25)	42.9	4 (2-12)
Cattle breeders (n=36)	46 (27-66)	75 (60-91)	20-16	19.4	15 (5-30)	38.9	1.5 (1-5)
P value	0.12	0.94	0.06	0.56	1	0.75	0.1

Table 3-3 shows the distribution of total white blood cell counts and differential between breeders and non breeders, while in **Table 3-4** the distribution among cattle and swine breeders is shown. All the parameters were in the range of reference values for all the workers, and no significant difference between groups was found, with the exception of an increase in the percentage of eosinophils in cattle breeders.

Table 3-3. White blood cell count of agricultural workers (breeders/non breeders) (n=96)

Variable	All Breeders (n=64)		Non -breeders (n=32)		P value
	Median	Min-Max	Median	Min-Max	
WBC (10^3/µl)	6.65	3.54-11.19	7.0	4.28-11.21	0.94
Neutrophils (%)	56.4	40.1-74.9	58.3	36.5-68.9	0.74
Lymphocyte (%)	33.2	16.4-48.6	30.10	20.5-54.4	0.73
Monocyte (%)	7.9	4.2-13.6	8.00	5-9.8	0.92
Eosinophil (%)	2.3	0-9	1.7	0-11	0.097
Basophil (%)	0.3	0-1.3	0.3	0.1-1.3	0.20

Table 3-4. White blood cell count of pig breeders and cattle breeders (n=96).

Variable	Pig Breeders (n = 28)		Cattle Breeders (n=36)		P value
	Median	Min-Max	Median	Min-Max	
WBC (10^3/µl)	6.53	4.65-11.99	6.8	3.54-10.76	0.82
Neutrophils (%)	59.15	42.4-74.9	54.8	40.1-72.5	0.26
Lymphocyte (%)	32.35	16.6-44	34.3	16.4-48.6	0.49
Monocyte (%)	7.95	4.2-13.6	7.9	5.1-12.3	0.64
Eosinophil (%)	2.15	0-8	2.80	0-9	**0.019**
Basophil (%)	0.25	1-1.20	0.3	0.0-1.3	0.67

In the same subjects, serum cytokines were assessed by commercially available ELISA kit. In **Table 3-5** and **Table 3-6** the serum levels of IL-6, IL-8, IL-10, TNF- α and IFNγ in breeders and non-breeders, and among pig and cattle breeders are reported. As shown in **Table 3-5**, in comparison to control subjects, increased median levels of TNF-α, IL8 and IL-10 were observed in animal breeders and the difference of these parameters were statistically significant between these two groups whilst median serum levels of IL-6 and IFNγ did not significantly change.

Table 3-5. Immune serum parameters between breeders and non breeders (control group) (n=96).

Variable	Animal breeders (n = 64)		Non breeders (n=32)		P value
	Median (pg/ml)	Min-Max	Median (pg/ml)	Min-Max	
IFN-γ	10.8	7.8-41.8	10.5	8.6-16.7	0.287
TNF-α	190.1	113.8-18076.9	147.7	39.1-9858.2	**0.001**
IL-10	44.4	26.3-9481.1	34.6	27-6515.1	**0.006**
IL-8	41.3	27.9-168.4	31.7	14.6-55.3	**0.000**
IL-6	5	3.6-142.8	4.55	3.4-7.5	0.104

Among the two breeder subgroups, a statistically significant difference in the median serum concentrations of TNF-α and IL-10 is observed, with the highest levels found in the swine breeders (**Table 3-6** and **Figure 3-1**).

Table 3-6. Immune serum parameters between swine breeders and cattle breeders (control group) (n=96).

Variable	Swine breeders (n = 28)		Cattle breeders (n=36)		P value
	Median (pg/ml)	Min-Max	Median (pg/ml)	Min-Max	
IFN-γ	11	7.6-41.8	10.35	8.3-19.9	0.057
TNF-α	215.6	113.8-18076.9	171.55	122.1-17578.3	**0.045**
IL-10	78	27-9481.1	38.6	26.3-8587.5	**0.011**
IL-8	52.1	28.5-154.2	38.5	27.9-168.4	0.150
IL-6	5.2	4.1-142.8	4.8	3.6-80.4	0.179

In **Figure 3-1** the minimum values, the lower quartile (Q1), the median, the upper quartile (Q3) and the maximum values of the measured cytokines between breeders and non breeders are shown through box plots. Outliers and extreme values are represented with circles (°) and asterisks (*), respectively.

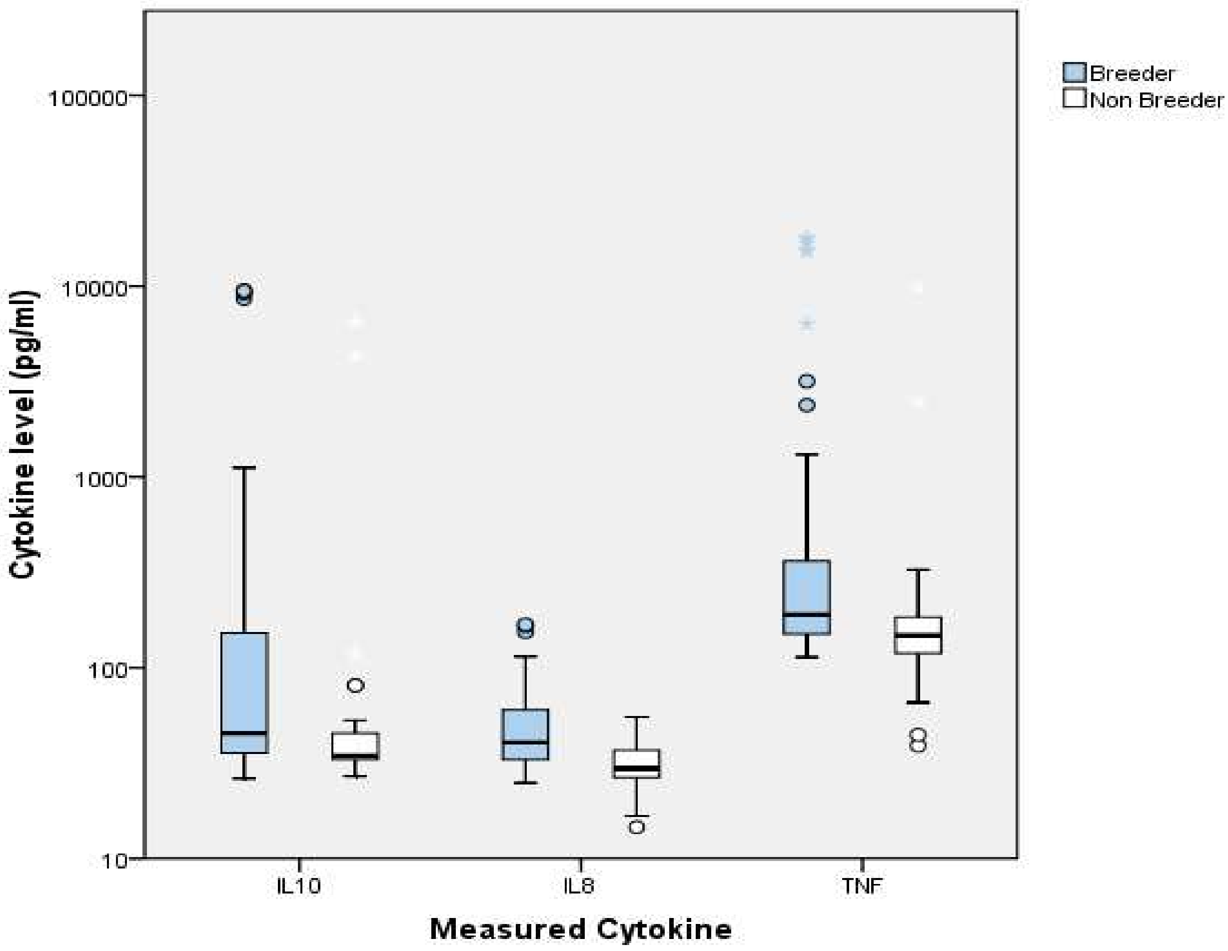

Fig.3-1. Serum cytokines in animal breeders and non breeder workers.

No significant correlation was found between serum proteins and serum cytokines. Overall, data presented shows that the serum levels of the proinflammatory cytokines TNF-α and IL-8, and the anti inflammatory cytokine IL-10 were significant increased in animal breeders.

3.1.3 DISCUSSION

The main finding of this pilot study was a statistically significant increase in the serum concentrations of TNF-α, IL-8 and IL-10 in animal breeders compared with "non-breeders" together with a slight increase in α1-globulin, β-globulin and total serum proteins, while no changes were observed in serum levels of IL-6 and IFN-γ or white blood cell counts. Altogether, our findings suggest that breeders showed a condition of immune system activation, and that this condition is particularly evident in swine workers. These findings are consistent with literature data, showing that organic dusts exposure in swine breeders represents a respiratory health hazard (Larsson *et al*., 1992; Palmberg *et al*., 2002; Von Essen and Romberger, 2003; Kirkhorn and Garry, 2000). Several studies report an increase of the number of inflammatory cells, predominantly neutrophilic granulocytes in the deep lung, as assessed by bronchoalveolar lavage, and a release of TNF- α, IL-6 and IL-1β in the airways in swine farmers (Wang *et al.*, 1997; Vogelzang, 1999). Also an increase of serum levels of IL-6 and TNF-α was observed in healthy subjects as a consequence of a few hours exposure to swine breeding farm dusts. In some studies, the increase of serum interleukins was observed within few hours after exposure (beginning versus end of shift) (Donham *et al*, 1984; Asmar *et al* 2001). Interestingly, such changes were more prominent in winter, when the windows of the breeding farms are closed, strongly suggesting the role of indoor environmental contaminants, in particular organic dusts and endotoxin (Bønløkke *et al*, 2009; Asmar *et al.*, 2001; O'Shaughnessy *et al*., 2002).

The increase of cytokines such as TNF-α and IL-8 and airway inflammation has been reported in some studies (O'Shaughnessy *et al.*, 2002; Pedersen *et al*, 1996). In addition, exposure to swine dusts has been shown to cause symptoms such as fever, headache and malaise accompanied by intense airway inflammation and influx of inflammatory cells into the upper and lower airways (Wang *et al.*, 1997; Cormier *et al*, 1997, Wang *et al.*, 1998). Our data indicate that the observed changes, in the absence of sign of health impairment, might be indicative of a risk of evolution into airway inflammation. Increased serum cytokines might be interpreted as immune changes indicative of lung inflammation that may potentially evolve into overt disease, such as ODTS (Rushton, 2006).

Several studies proved that serum protein concentrations may be altered as a result of different disease states and interpretation of serum protein electrophoretic patterns can be helpful in confirming the diagnosis of some diseases (Israël-Assayag and Cormier 2002). Our results showed a statistically significant difference between animal breeders and non breeders serum concentration of α1, β-globulin and total serum protein. This might be due to increased exposure to biological agents in animal breeders (e.g. acute/ chronic infections etc.). In this study increase in serum proteins seems consistent with increased serum cytokines. A difference among pig and cattle breeders was observed. Bigger changes were found in the swine breeders. This may be due to the phase of pig weighing, which is typically associated with a high degree of agitation and aero-solization of and exposure to air-borne dusts. Furthermore, air sampled from pig confinement buildings contains grain dusts, ammonia, fungi and bacteria, mostly Gram-positive but also Gram-negative bacteria, typically related with ODTS.

The role of airborne endotoxins in modulating these immune changes is under discussion. Another study showed that pig farmers with less than 5 years of work had a higher prevalence of organic dust toxic syndrome than those who had been working as farmers for a longer time, suggesting the possibility of an adaptation mechanism (Von Essen *et al.*, 2003).

Endotoxin might play a role also in this adaptation, since it is known that repeated exposure to these substances may results in an attenuation of the inflammatory response, referred to as “endotoxin tolerance” (Cavaillon, 1995; Israël-Assayag and Cormier, 2002). This may be associated with the increased level of IL-10 we observed in animal breeders. Moreover, it should be taken in due account that animal feedings contain high amounts of proteins from soybean in which specific mitogens such as lectins can be present, and also the immune reactivity of pollens should be taken into consideration.

3.2 Spirometry Test

3.2.0 Introduction and aim of the study

Since working in animal housings can be associated with exposure to organic dust, bacteria, moulds, endotoxin, and ammonia in concentrations that can induce cellular and immunological responses and result in respiratory diseases, respiratory symptoms are commonly reported among farmers and animal breeders, particularly in swine farmers.

For this reason, we decided to evaluate all the spirometric tests that were conducted during routine health surveillance for the two study groups to check whether immune changes are associated with signs of respiratory impairment. Spirometric measures of lung function were compared between breeders and farmers as control subjects.

3.2.1 Methods

In both groups of the study subjects (Groups A and B, n=199) pulmonary function data were collected according to the standard protocol with a portable Spirometer, (Pony Fx®, COSMED® equipment, USA). All subjects were at rest for 15 minutes and were not allowed to smoke for 1 hour prior to the measurement. The procedure was carefully explained to the agricultural workers to avoid leaks around mouthpiece and to make a maximum inspiration with perfect expiration and without hesitation and leaning forward. The registered parameters were FVC, FEV1, PEF, PIF, FEF 25-75%, MEF 75%, MEF 50% and MEF 25%. To avoid cross infection with microorganisms between subjects, disposable bacterial filters (Micro Gard, Viasys Healthcare) were used. At least three satisfactory forced maximal expirations were performed by each subject and the best value was accepted for analyses. The results were also expressed as a percentage of predicted values and FEV1/FVC x 100 was calculated.

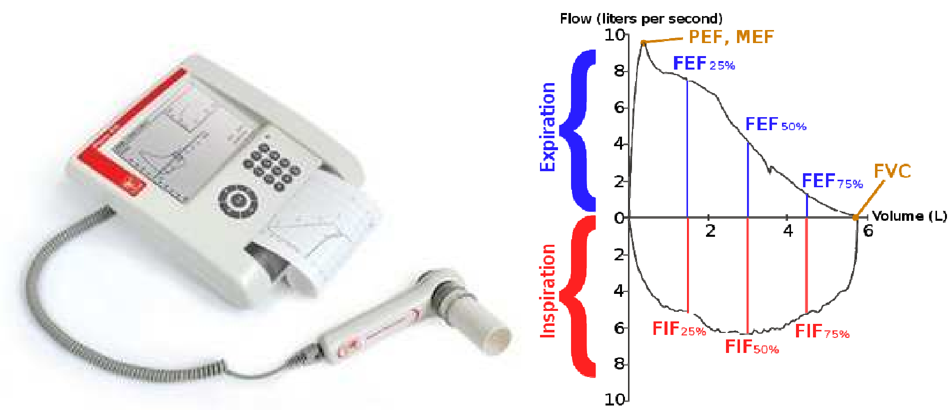

Fig 3-2. Pony Fx Desktop Spirometer and Flow-Volume loop showing successful FVC maneuver.

Data Analysis

We used Statistical Package for Social Sciences SPSS (version 18) and STATA package (version 12). Since the distribution of values were normal, mean values and standard deviations (SDs) for all the lung function measures for the 199 participants were calculated. Association between measured lung function parameters and exposure among the 199 participants were estimated in crude and adjusted additive differences in linear regression models. Results of linear regression were presented as odds Ratios (OR) with 95% confidence intervals (CI). Odds Ratios were adjusted for the potential confounders (age, nationality, job and smoking).

3.2.2 Results

Majority of workers had normal spirometry results (83.2%) as illustrated in **Table 3-7** below.

Table 3-7. Spirometry results for the sample of workers (2010-2012)

PFR Results	Percentage
Normal	83.2
Obstructive	2.4
Restrictive	5.7
Not interpretable	7.7
Small airways obstruction	1.0
Total	100.0

Tables 3-8 to 3-10, explain about lung function indices resulted from spirometry tests for both groups of workers. Mean, crude and adjusted values for univarite and multivariate linear regression models were calculated and presented in the following tables.

Table 3-8. Lung function indices (as percent of theoretical) by job title. Results of univariate and multiple linear regression models (Group A, n=103).

	No.	Mean	Crude difference	95% CI	Adjusted difference*	95% CI
FVC						
Farmer	12	98.5	(Reference)		(Reference)	
Breeder	63	99.3	1.4	-9.8,12.7	4.0	-8.4,16.4
Swine	*23*	*96.7*	*-1.1*	*-13.9,11.7*	*0.0*	*-14,14*
Cattle	*30*	*99.4*	*1.5*	*-10.7,13.8*	*4.6*	*-9.2,18.3*
Fish	10	104.9	7.0	-8.4,22.4	11.8	-6.3,29.8
FEV1						
Farmer	12	93.8	-	(Reference)	-	(Reference)
Breeder	61	94.7	1.94	-7.9,11.7	4.7	-5.8,15.3
Swine	*21*	*97.1*	*3.3*	*-8,14.7*	*5.1*	*-6.9,17.1*
Cattle	*30*	*96.3*	*2.5*	*-8.2,13.2*	*7.2*	*-4.4,18.8*
Fish	10	91	-2.8	-16.2,10.6	-2.5	-17.8,12.7
PEF						
Farmer	12	85.2	-	(Reference)	-	(Reference)
Breeder	63	79.8	-5.5	19.3,8.4	-5.4	-20.1,9.4
Swine	*23*	*69.3*	*-14.5*	*-29.2,0.3*	*-12.7*	*-27.9,2.5*
Cattle	*30*	*89*	*3.4*	*-10.7,17.6*	*6.3*	*-8.6,21.2*
Fish	10	81.1	-11.6	-29.4,6.2	-17.9	-37.5,1.6
FEV1/FVC %						
Farmer	12	97.5	-	(Reference)	-	(Reference)
Breeder	*61*	*99.7*	1.2	-8.0,10.4	4.4	-6.1,15.01
Swine	*21*	*97.8*	*-0.7*	*11.4,9.9*	*0.8*	*-11.3,12.9*
Cattle	*30*	*100.9*	*2.3*	*-7.7,12.4*	*7.12*	*-14.5, 18.8*
Fish	10	100.4	1.9	-10.7,14.5	5.35	-9.9,20.6
FEF25-75%						
Farmer	*12*	*83.7*	-	(Reference)	-	(Reference)
Breeder	*62*	*82.2*	*-0.5*	*-17.5,16.5*	*3.3*	*-14.7,21.2*
Swine	*22*	*89.1*	*5.4*	*-13.9,24.9*	*4.9*	*-15.7,26.5*
Cattle	*30*	*81.1*	*-2.6*	*-21,15.9*	*3.7*	*-16.4,23.8*
Fish	10	76.4	-7.3	-30.5,15.8	-1.5	-27.9,24.8
MEF 75%						
Farmer	*12*	*84.9*	-	(Reference)	-	(Reference)
Breeder	*53*	*77.5*	*-7.4*	*-21.5,6.8*	*-5.5*	*-20.4,9.4*
Swine	*21*	*71.8*	*-13*	*-28.6,2.5*	*-11.4*	*-27, 4.9*
Cattle	*25*	*85*	*0.13*	*-15,15.2*	*2.4*	*-13.4,18.4*
Fish	7	67.6	-17.20	-37.7,3.3	-12.8	-33.7,8.2
MEF 50%						
Farmer	*12*	*99.4*	-	(Reference)	-	(Reference)
Breeder	*53*	*87.5*	*-2.4*	*-19.4,14.5*	*-1.3*	*-19.3,16.7*
Swine	*21*	*89.4*	*-0.4*	*-19.9,19.1*	*-0.8*	*-21.5,19.9*
Cattle	*25*	*86.1*	*-3.8*	*-22.7,15.1*	*-2.2*	*-22.4,17.9*
Fish	7	86.3	-3.5	-29.1,22.5	-0.9	-26.5,26.4
MEF 25%						
Farmer	*12*	*89.6*	-	(Reference)	-	(Reference)
Breeder	*53*	*88.7*	*7.5*	*-15.2,30.2*	*12.7*	*-10.1,35.6*
Swine	*21*	*98.5*	*16.8*	*-8.8,42.5*	*17.8*	*-8.3,43.9*
Cattle	*25*	*84.1*	*2.4*	*-22.4,27.3*	*9.3*	*-16.1,34.7*
Fish	7	79.1	-2.7	-36.2,31.1	10.3	-23.1,43.7

***Adjusted for age, country of origin (EU vs non-EU), smoking (yes, no; and cigarettes/day).**

Table 3-9. Lung function indices (as percent of theoretical) by job title. Results of univariate and multiple linear regression models (Group B, n=96).

	No.	Mean	Crude difference	95% CI	Adjusted difference*	95% CI
FVC						
Farmer	26	98.3	(Reference)		(Reference)	
Breeder	54	97.1	-1.2	-7.7, 5.3	1.6	-5.1, 8.4
Swine	*24*	*98.8*	*0.5*	*-7.2, 8.2*	*2.2*	*-5.8, 10.1*
Cattle	*30*	*95.7*	*-2.6*	*-9.9, 4.7*	*1.2*	*-6.3, 8.8*
FEV1						
Farmer	27	98.2	-	(Reference)	-	(Reference)
Breeder	55	93.9	-4.3	-11.5, 2.9	-0.6	8.8, 7.6
Swine	*24*	*95.6*	*-2.6*	*-11.3, 6.0*	*0.4*	*-9.1, 10.0*
Cattle	*31*	*92.7*	*-5.6*	*-5.6, 4.1*	*-1.3*	*-10.4, 7.7*
PEF						
Farmer	26	91.0	-	(Reference)	-	(Reference)
Breeder	53	81.9	-9.1	-20.4, 2.25	-6.8	-19.7, 6.1
Swine	*24*	*82.3*	*-8.7*	*-22.1, 4.8*	*-7.9*	*-23.1, 7.18*
Cattle	*29*	*81.6*	*-9.4*	*-22.3, 3.4*	*-5.9*	*-20.3, 8.5*
FEV1/FVC %						
Farmer	27	104.2	-	(Reference)	-	(Reference)
Breeder	*55*	*100.6*	-3.6	-9.0, 2.1	0.27	-5.9, 6.5
Swine	*24*	*100.1*	*-4.1*	*-11.0, 2.7*	*-1.3*	*-8.6, 5.9*
Cattle	*31*	*101.0*	*-3.2*	*-9.6, 3.2*	*1.5*	*-5.3, 8.4*
FEF25-75%						
Farmer	*26*	*97.2*	-	(Reference)	-	(Reference)
Breeder	*48*	*85.0*	*-12.2*	*-26.8, 2.4*	*-6.1*	*-21.9, 8.3*
Swine	*20*	*86.4*	*-10.7*	*-28.7, 7.2*	*-6.1*	*23.9, 11.6*
Cattle	*28*	*83.9*	*-13.2*	*-29.7, 3.2*	*-7.3*	*24.2, 9.4*
MEF 75%						
Farmer	*25*	*97.2*	-	(Reference)	-	(Reference)
Breeder	*47*	*85*	*-12.2*	*-26.8, 2.4*	*-6.8*	*-21.9, 8.3*
Swine	*20*	*82.3*	*10.8*	*-28.7, 7.2*	*-6.1*	*-23.9, 11.6*
Cattle	*27*	*83.9*	*-13.2*	*-29.7, 3.2*	*-7.4*	*-24.2, 9.5*
MEF 50%						
Farmer	*25*	*99.2*	-	(Reference)	-	(Reference)
Breeder	*47*	*88.1*	*-11.1*	*-25.7, 3.6*	*-6.6*	*-22.4, 9.2*
Swine	*20*	*88.6*	*-10.6*	*-28.5, 7.2*	*-6.3*	*-24.7, 12.1*
Cattle	*27*	*87.8*	*-11.4*	*-27.9, 5.8*	*-6.9*	*-24.5, 10.8*
MEF 25%						
Farmer	*25*	*107.9*	-	(Reference)	-	(Reference)
Breeder	*47*	*85.9*	*-16.3*	*-42.5, -1.45*	*-16.3*	*-37.8, 5.2*
Swine	*20*	*87.6*	*-20.4*	*-45.4, 4.7*	*-15.2*	*-40.3, 9.8*
Cattle	*27*	*84.7*	*-23.2*	*-46.4, -0.3*	*-17.1*	*-41.2, 6.9*

***Adjusted for age, country of origin (EU vs non-EU), smoking (yes, no; and cigarettes/day) and BMI**

Table 3-10. Lung function indices (as percent of theoretical) by job title. Results of univariate and multiple random-intercept linear regression models (Groups A and B, n = 199).

Test/ worker	No.	Mean	Crude difference	95% CI	Adjusted difference*	95% CI
FVC						
Farmer	39	98.1	(Reference)		(Reference)	
Breeder	119	98	1.1	-4.8, 6.9	3.1	-2.8,9.0
Swine	48	98.7	0.6	-6.4, 5.6	1.2	-5.6,8.0
Cattle	61	96.3	0.2	-6.3, 6.7	3.1	-3.5,9.7
Fish	10	104.9	7.7	-3.2, 18.7	13.1	0.8,25.5
FEV1						
Farmer	40	97.3	-	(Reference)	-	(Reference)
Breeder	118	94.5	0.5	-5.2,6.1	3.0	-2.8,8.8
Swine	46	97.3	2.3	-4.8,9.2	3.3	-3.7,10.5
Cattle	62	92.9	0.0	-6.3,6.3	3.6	-2.8,10.0
Fish	10	90.9	-3.5	-14.2,7.2	-2.9	-15.4,9.6
PEF						
Farmer	39	89.3	-	(Reference)	-	(Reference)
Breeder	118	79	-8.1	-16.2,-0.0	-5.8	-14.3,2.8
Swine	48	77.8	-11.1	-21,-1.1	-9.5	-19.4,0.5
Cattle	60	83.9	-5.2	-14.1,3.6	-1.0	-10.4,8.4
Fish	10	73.6	-15.3	-30.9,0.2	-19.4	-37.5,-1.4
FEV1/FVC %						
Farmer	40	102.9	-	(Reference)	-	(Reference)
Breeder	118	100.1	-3.2	-8.0,1.6	-1.2	-6.6,4.0
Swine	46	99.2	-4.0	-9.9,1.9	-3.1	-9.4,3.3
Cattle	62	100.7	-2.8	-8.1,2.5	-0.1	-6.1,5.9
Fish	10	100.4	-2.6	-11.8,6.6	0.3	-11.1,11.6
FEF25-75%						
Farmer	39	94.5	-	(Reference)	-	(Reference)
Breeder	112	83	-10.1	-19.8,-0.5	-3.5	-13.5,6.5
Swine	43	89.3	-2.5	-15.3,10.3	0.6	-12.4,12.5
Cattle	59	79.6	-12.8	-23.3,-2.4	5.4	-16.5,5.8
Fish	10	76.3	-16.3	-35.6,3.0	-7.6	-29.7,14.6
MEF 75%						
Farmer	38	92.4	-	(Reference)	-	(Reference)
Breeder	102	80	-11.4	-20.3,-2.6	-6.6	-15.6,2.4
Swine	42	78.2	-13.1	-24.2,-2.0	-9.3	-19.9,1.4
Cattle	53	83	-8.9	-18.6,0.7	-2.9	-12.9,7.0
Fish	7	67.6	-23.9	-43.1,-4.8	-17.3	-36.3,1.6
MEF 50%						
Farmer	38	97.6	-	(Reference)	-	(Reference)
Breeder	102	86.8	-8.1	-18.2,1.9	-3.5	-13.9,6.8
Swine	42	90.4	-4.4	-17.4,8.5	-1.5	-14,11.1
Cattle	53	83.8	-10.1	-21.3,1.1	-5.2	-16.9,6.5
Fish	7	86.3	-9.1	-31.4,13.2	-3.4	-25.5,18.8
MEF 25%						
Farmer	38	101.1	-	(Reference)	-	(Reference)
Breeder	102	86.3	-15.6	-30,-1.3	-4.9	-19.2,9.4
Swine	42	93.9	-6.3	-23.8,11.2	0.9	-15.8,17.7
Cattle	53	81.2	-20.9	-20.9,8.1	-9.9	-25.9,6.1
Fish	7	79.1	-22.1	-22.1,15.6	-4.2	-34.1,25.6

***Adjusted for age, country of origin (EU vs non-EU), smoking (yes, no; and cigarettes/day)and BMI.**

3.2.3 Discussion

Different studies have shown that longer exposure to swine confinement environment can cause more decline in FVC and FEV1 and also accelerate mean age related annual decline in FEV1 (Omland, 2002; Djuricić *et al.*, 2004; Zedja *et al.*, 1993). Our results implied that although animal breeders had shown relatively lower mean values of spirometry measurements than farmers but this difference was not statistically significant. The other point was that there was a statistically significant difference between European and Non-European workers for FVC, FEV1, PEF, FEF 25-75%, MEF 75% and MEF 25% through linear multivariate regression analysis which is consistent with the literature. Studies in other countries suggest that those of African, Japanese, Indian, and other non-European ethnicities have lower lung function for their body size than Europeans (Hancox and Baxter, 2006; Pellegrino *et al.*, 2005). In these countries, predicted values for non-whites are sometimes adjusted by subtracting an “ethnic factor” of 12% from the predicted values for Europeans. While most of the LFT studies performed in non-European populations have shown greatly decreased lung volumes compared to Europeans.

Finally, the present study did not point out any significant difference in the values of the basic pulmonary function tests in the animal breeders vs. controls. This might suggest that, despite some evidence of immune activation in workers and in particular animal breeders, there is not a clear evidence of respiratory effect suggesting that the levels of activation observed can be considered adaptive and not early signs of disease. Further investigation on this issue is recommended.

Chapter Summary

In this chapter the third pilot study has been explained. In the last step a spirometry test has been carried out to compare the results between two types of workers (exposed/ control). In the following chapter final conclusions and recommendations of the studies are presented.

CHAPTER IV Overall Conclusions

This PhD project has been carried out in the frame of a wide research project that is being conducted in the region of Lombardy, addressed at occupational exposure to biological risk factors among farmers and animal breeders. Therefore, the overall results of these studies consit of a broad information regarding i) the exposure of workers to a selected group of biological agents (bacteria, viruses and organic dusts); ii) the identification of early immunological signs of effect and iii) the assessment of the immunity against tetanus of the agricultural workers of the region, considering different population subgroups. The overall results of the studies have brought the following integrated conclusions:

High percentage of anti *Coxiella* and *Leptospira* antibodies found in agricultural workers might suggest that these agents might be widespread in north Italy. There was no anti-*Brucell*a and *Salmonella* IgG detected which might reflect the fact that this area is clear from these two important zoonotic diseases. Our results suggest that zoonoses, especially *Coxiella* and *Leptospira* in north Italy may have a work related character and all agricultural workers (breeders and farmers) irrespective of contact to animals, are accounted as at risk groups because of the ubiquitous presence of these risk factors in the rural environment (for example, *leptospira* in surface water contaminated by rodents' urine) while some tick-borne diseases (*Lyme borreliosis*) which can also affect farmers are probably underestimated, perhaps due to the low access of agricultural workers to occupational health care and a still poor surveillance of these diseases. Finally, the present data draw attention to the need to confirm and extend seroprevalence studies in a broader high risk population in order to better define the risk and, if necessary, take appropriate measures to prevent zoonotic diseases.

With regards to anti HEV IgG method, we compared two type of commercial assays which resulted in different outcomes in a cohort of workers, and one out of the two showed an unanticipated high prevalence on antibodies in the study groups, confirming that hepatitis E can be considered an emerging threat for pig breeders. However, it is also shown by literature that different assays for the detection of anti-HEV IgG can result in significantly different results. Therefore, future studies on the development of standard diagnostic tests and their validation are warranted.

Farmers in our region had relatively good immune status to tetanus and in general Italian and other workers from Eastern European countries had better immunity in comparison to migrant workers from developing countries. Occupational health physicians and other health personnel should have clear that investigation on history of immunization especially, in migrant workers, can fail in providing reliable information but that very often anamnesis collection alone overestimate the real risk, since most of the workers have some, even very small, coverage. In this light, preventing tetanus should be a high priority for all primary care physicians and occupational health personnel, having in mind that in the majority of the cases a booster dose of tetanus toxoid is extremely safe and effective in providing the coverage needed in all the cases where, for several reasons, the detection of antibody titer cannot be performed.

Different studies have shown that occupational exposure to organic dusts induces inflammatory responses and apparent condition of immune system activation measurable in serum levels, which might evolve to ODTS. In this pilot study, we found that animal breeders show signs of immune system activation, and that pig breeders are a subgroup particularly at risk. Deeper investigations especially in pig breeding facilities, involving higher numbers of workers, collecting key environmental parameters, such as quantity and quality of the main indoor airborne contaminants are necessary. The prognostic significance of the observed changes may be clarified through the collection of retrospective epidemiological data as well as during the continuous health surveillance of animal breeders, with a particular attention to any immune system and respiratory system changes.

It is noteworthy to underline that spirometry results failed to point out any significant difference among animal breeders and controls regarding lung functions, and this data might suggest that for the levels of immune activation observed in the study, no significant respiratory changes are anticipated. On the other hand, spirometry study confirmed that there were statistically significant differences between workers by their nationality, as expected by literature.

Recommendations and Dissemination of the Study Results

As in any other study on biological risks and animal breeding, a close collaboration between medical doctors and veterinarians is strongly needed, in the frame of the so called "one health approach". Since animal breeders are presumably the population subgroup mostly exposed to biological agents, they must be firstly addressed by studies, having in mind that also consumers have to be taken into account. It is also clear that food quality and safety can't be assured if workers' and animals' health as well as welfare are not equally implemented. In this framework workers employed in each phase of the productive process, from breeding to slaughtering, have to be considered. As a matter of fact, they can as well act as active or passive subject in the transmission of diseases: workers can be infective, due to a contagious status, or infected by pathogenic agents able to cause from mild to severe diseases.

The findings resulting from this research might have economical and sanitary implications for new management and food tracking procedures, which includes reductions of farming cost, decrease of zoonotic disease risks and customer safety

The results of the study might be beneficial for the improvement of knowledge on the biological (bacteria and viruses) and chemical (organic dusts) risks present in specific stages of the animal breeding and food production chain (breeding, slaughtering, processing) and their mechanisms of action, and might help occupational health physicians in setting up their health surveillance programmes. This will allow to highlight the different phases of the production cycle in which specific risks are generated, and to identify the objectives of appropriate preventive interventions. The intense activities of dissemination of the results can be spread among farmers, consumers and other stakeholders (e.g. veterinarians and physicians, etc.) for the purpose of awareness of the possible risk factors present in the sector together with appropriate tools for their prevention and control. In addition results of the current study were presented to the scientific communities during national and international congresses and also to the various social actors interested in regional events, and finally submitted to international journals for publications.

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Appendix I Study data sheet & consent form

PROGETTO:

"PRESENZA E DISTRIBUZIONE DI INDICATORI DI CONTATTO CON IL VIRUS DELL'EPATITE E E DI ALTRI AGENTI BIOLOGICI NEGLI ALLEVATORI DELLA REGIONE LOMBARDIA"

SCHEDA INFORMATIVA

Presso l'Azienda Ospedaliera San Paolo, Polo Universitario, è in corso un progetto di ricerca in stretta collaborazione con l'Università degli Studi di Milano che ha lo scopo di studiare la sieroprevalenza di anticorpi indicatori di contatto con il virus dell'epatite E ed altri agenti biologici patogeni nei lavoratori degli allevamenti della Lombardia.

I partecipanti allo studio saranno reclutati su base volontaria negli allevamenti lombardi, a partire da coloro che sono già coinvolti nel programma di sorveglianza sanitaria realizzato dall'Azienda Ospedaliera San Paolo, Polo Universitario.

Lo studio trasversale ha lo scopo di definire la prevalenza di specifici indicatori di avvenuto contatto con patogeni di origine animale e, in particolare, virus dell'epatite E e verificare possibili variazioni del rischio in rapporto alla tipologia di allevamento ed alla mansione svolta.
Questo studio porterà evidenti benefici per i lavoratori degli allevamenti, verso i quali potrebbero essere avviati specifici programmi di prevenzione, indirizzati a ridurre il rischio di contatto con agenti biologici e quindi anche gli effetti più gravi, quali ad esempio i casi di epatite E fulminante che colpiscono soprattutto le donne in gravidanza. Altri benefici effetti dello studio potranno interessare gli animali degli allevamenti e, soprattutto, i consumatori di cibi di origine animale. Ovviamente, i partecipanti avranno anche il beneficio di un approfondito controllo del proprio stato di salute.

Per svolgere la ricerca descritta è necessaria la collaborazione di lavoratori disponibili a sottoporsi a:

1. Intervista sullo stato di salute e sul lavoro svolto dalla fine delle scuole;
2. Visita medica generale;
3. Un unico prelievo di sangue venoso per la ricerca degli anticorpi previsti dal protocollo.

Il tutto sarà effettuato da un Medico abilitato e richiederà non oltre mezzora di tempo.

Non sono previsti rischi, né effetti collaterali o disagi essendo il prelievo venoso una procedura poco invasiva

Prima di accettare potrà consultarsi con i suoi famigliari o con il suo medico di base, se lo ritiene necessario.
La informiamo che i suoi dati personali, ai sensi del D. L. n'196/2003, verranno raccolti ed archiviati in modo adeguato e saranno usati esclusivamente allo scopo della ricerca in forma anonima.

La sua partecipazione allo studio è assolutamente libera e volontaria. Potrà decidere di non partecipare o di ritirare il consenso in qualsiasi momento e per qualsiasi ragione, senza che ciò comporti alcuna riduzione nell'attenzione dei Suoi confronti.

Con il Suo consenso, il Medico di famiglia verrà informato della Sua partecipazione allo Studio e potrà richiedere al Medico sperimentatore informazioni dettagliate al riguardo.

Le chiediamo infine di poter impiegare i dati scientifici che emergono dal presente studio, ferma restando l'assoluta riservatezza dei Suoi dati personali, in accordo con la normativa vigente (Legge n. 196/2003).

Il Protocollo di questo esperimento è stato redatto in conformità alle indicazioni della Dichiarazione di Helsinki e delle orme di Buona Pratica Clinica ed è stato approvato dal Comitato Etico di questo Ospedale.

Per qualunque informazione ulteriore e comunicazioni durante lo studio sarà a sua disposizione e potrà contattare il Dott. Colosio Claudio al numero telefonico 0281843465.

Firma del Medico Ricercatore
Dott. Claudio Colosio

CONSENSO ALLA PARTECIPAZIONE ALLO STUDIO

NOME E COGNOME	
INSEDIAMENTO PRODUTTIVO	
INDIRIZZO	
NUMERO DI TELEFONO	

1. Confermo di aver capito le informazioni fornite sulla sperimentazione propostami e di avere avuto l'opportunità di fare domande durante il colloquio informativo svoltosi in data odierna.

2. Capisco che la mia partecipazione è volontaria e che sono libero/a di ritirarmi in qualsiasi momento, senza dare alcuna spiegazione, senza che la mia assistenza medica e senza che i miei diritti legali vengano interessati.

3. Capisco che la mia cartella clinica possa essere letta dai ricercatori responsabili dello studio "PRESENZA E DISTRIBUZIONE DI INDICATORI DI CONTATTO CON IL VIRUS DELL'EPATITE E E DI ALTRI AGENTI BIOLOGICI NEGLI ALLEVATORI DELLA REGIONE LOMBARDIA".

4. Do l'autorizzazione perché questi ricercatori, guidati dal Dott. Claudio Colosio, abbiano accesso alla mia cartella clinica.

5. Acconsento a partecipare al suddetto studio.

__________________________ _________________ _______________________

Cognome/Nome Lavoratore   Data   Firma del partecipante

____________________ _________________ _______________________

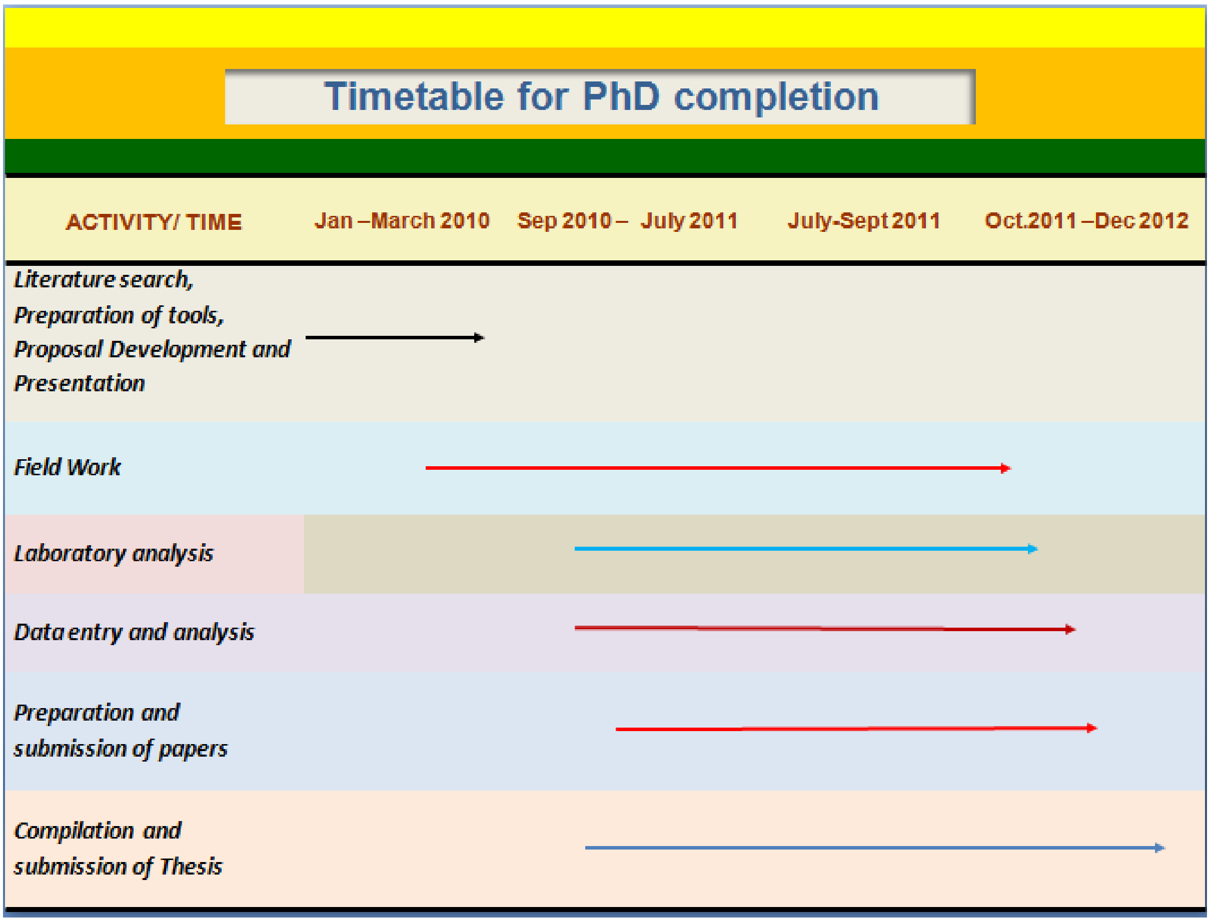
Cognome/Nome Medico   Data   Firma dello sperimentatore

che ha informato il paziente

Firma del testimone (obbligatoria solo quando il paziente non è in grado di leggere il modulo di consenso informato)

Firma del Rappresentante legale (obbligatoria solo nei casi previsti dalla legge)

Appendix II PhD Timetable



Appendix III Table of Main zoontic diseases

Main Zoonotic Diseases; Source Health Protection Agency (Source: HPA)

Disease	Organism	Main reservoirs	mode of transmission to humans
Anthrax	Bacillus anthracis	livestock, wild animals, environment	direct contact, ingestion
Animal influenza	influenza viruses	livestock, humans	may be reverse zoonosis
Avian influenza	Influenza virus, avian strains	poultry, ducks	direct contact
Bovine tuberculosis	Mycobacterium bovis	cattle	milk
Brucellosis	Brucella species	cattle, goats, sheep, pigs	dairy products, milk
Cat scratch fever	Bartonella henselae	cats	bite, scratch
Cysticercosis	*Taenia* species	cattle, pigs	meat
Cryptosporidiosis	Cryptosporidium species	cattle, sheep, pets	water, direct contact
Erysipeloid	Erysipelothrix rhusiopathiae	pigs, fish, environment	direct contact
Fish tank granuloma	*Mycobacterium marinum*	fish	direct contact, water
Food poisoning	Campylobacter species	poultry, farm animals	raw meat, milk
	Salmonella species	poultry, cattle, sheep, pigs	foodborne
Giardiasis	Giardia lamblia	humans, wildlife	waterborne, person to person
Glanders	Burkholderia mallei	horse, donkey, mule	direct contact
Haemorrhagic colitis	Escherichia coli O157	ruminants	direct contact (and foodborne)
Hantavirus syndromes	Hantaviruses	rodents	aerosol
Hepatitis E	Hepatitis E virus	not yet known	not yet known
Hydatid disease	Echinococcus granulosus	dogs, sheep	ingestion of eggs excreted by dog
Leptospirosis	Leptospira species	rodents, ruminants	infected urine, water
Listeriosis	Listeria monocytogenes	cattle, sheep, soil	dairy produce, meat products
Lyme disease	Borrelia burgdorferi	ticks, rodents, sheep, deer, small mammals	tick bite
Orf	Orf virus	sheep	direct contact
Pasteurellosis	Pasteurella multocida	dogs, cats, many mammals	bite/scratch, direct contact
Plague	Yersinia pestis	rats and their fleas	flea bite
Psittacosis	Chlamydophila psittaci	birds, poultry, ducks	aerosol, direct contact
Q fever	Coxiella burnetii	cattle, sheep, goats, cats	aerosol, direct contact, milk, fomites
Rabies	Rabies viruses	dogs, foxes, bats, cats	animal bite
Rift Valley fever	Rift Valley fever virus	cattle, goats, sheep	direct contact, mosquito bite
Ringworm	Dermatophyte fungi	cats, dogs, cattle, many animal species	direct contact
Tickborne encephalitis	Tickborne encephalitis virus	rodents, small mammals, livestock	tickbite, unpasteurised milk products
Toxoplasmosis	Toxoplasma gondii	cats, ruminants	ingestion of faecal oocysts, meat
Trichinellosis	*Trichinella spiralis*	pigs, wild boar	pork products
Viral haemorrhagic fevers	Ebola, Crimean-Congo HF, Lassa and Marburg viruses	variously: rodents, ticks, livestock, primates, bats	direct contact, inoculation, ticks
West Nile fever	West nile virus	wild birds, mosquitoes	mosquito bite
Zoonotic diphtheria	Corynebacterium ulcerans	cattle, farm animals, dogs	direct contact, milk

Appendix IV Curriculum Vitae

TABIBI, RAMIN

Personal Details

Gender: **Male** Civil Status: **Married**

Country & Place of Birth: **Iran** Date of Birth: **1965**

First nationality: **Iran, Islamic Republic of**

E-mail for correspondence: **tabibir@yahoo.com**

Areas of Expertise

Communicable Disease Epidemiology 6-10 years

Environmental Health Epidemiology 6-10 years

Research 6-10 years

Language Skills

Mother Tongue 1 : **Persian** Mother Tongue 2 : **-**

U.N. Proficiency Examination **Yes**

If yes, please indicate the language(s) & year(s) certificate was obtained **IELTS 2004, Band Score 6.5**

Working Languages	*Speaking*	*Reading*	*Writing*
English	**Advanced**	**Advanced**	**Intermediate**
Italian	**Intermediate**	**Basic**	**Basic**
Arabic	**Basic**	**Intermediate**	**Basic**

Education

Educational details

Year	*Name of Education Institution*	*Degree / Diploma*	*Title of degree/diploma and description of studies*
2004 2006	**University of York York, Yorkshire United Kingdom of Great Britain and Northern Ireland**	**M. Sc**	**Health sciences (Health servicses Research)**
1995 1998	**Tehran University of Medical sciences TehranIran**	**M.PH**	**Medical Parasitology-M.Sc**

1991 1994	**Ahwaz Jondishapour university of Medical Sciences Iran**	**B.Eng**	**Public Health**
1984 1987	**Ahwaz Jondishapour university of Medical SciencesIran (Islamic Republic of)**	**B. Sc.**	**Public Health**
		-	

Other educational details

Occupational Health

International Experience

Are you currently employed by an international organization as explained above? **Yes**

Organization:	*Duty Station:*	*From:*	*Grade:*
International Centre for Rural Health, Milan, Ital	**PhD student, Epidemiologist, researcher**	**2010**	
Organization:	Duty Station:	From: - To:	
University of Kent	**Occupational Health department**	**2007 - 2009**	
		-	
		-	

Additional information, if any: **I have been mostly out of Iran since 2004, living 6 years in the England and 3 years in Italy and also travelling to other countries, Canada, Africa, Asia European countries and working experience of WHO research projects all forced me to apply for a suitable job in WHO website.**

Do you have professional experience outside of your home country (e.g., short-term assignments, management responsibility, extensive business travel)? **Yes**

Please specify the country and field of work: **Iran, Ministry of Heath, CDC group, Sudan, WHO_ Shistosomiasis executive eradication methods Italy, International Centre for Rural Health , Milan**

Present and previous employment

Exact Title of Position held:	**Director of Malaria and Schistosomiasis control in Khouzestan province, Iran**

Brief description of duties and responsibilities:

Teaching at University of Kent
Managing Malaria- and Shistosomiasis control programes in all the districtts in

Khouzestan province.
Cooperation in national and international research project (e.g.WHO research projects)
Preparing necessary medication for prevention and treatment
Preparing necessary workshops for training personnels
Data processing and analysis
Attending in international and national workshops and congresses
Attending in WHO national and International courses and workshops

Additional Information

Relevant experience, living abroad, additional skills

• Iran's representative in the World Health Organisation East Mediterranean Countries (EMRO) Inter-country Meeting of National Programme Managers for the Prevention and Control of Schistosomiasis and Soil-transmitted Helminthiases, Sana'a, Yemen May 2003.

• Fellowship in Schistosomiasis Eradication and Control – WHO - Khartoum, Sudan, September 2001.

• Diploma Course of Malaria and Planning its Control – WHO Regional Training Centre in Bandar Abbas, Iran September and November 2000.

LECTURES

• Teaching experience in KIMHS, University of Kent September 2007.

• Established various courses in prevention and control of communicable diseases for health personnel in Khuzestan province from 1994-2004.

• Organised training seminars and workshops for the health staffs from 1994 - 2004.

HONOURS
• Commendation from AJUMS Vice Chancellor of Health Affairs for the research on the prevalence of iron deficiency anaemia in 6-59 month children of Ahwaz district in Iran, 2005.

• Commendation from the Minister of Health for participation in Poliomyelitis Eradication Programme 2003.

AWARDS

• Scholarship Award from department of Occupational Medicine, , "Clinica del Lavoro" of Università degli Studi di Milano for a 3 years PhD course in

Occupational Medicine and Industrial Hygiene from JAN 2010 to DEC 2012.

• Scholarship Award from the Iranian ministry of Health and Medical Education for an MSc in Health Services Research, University of York , United Kingdom. 2004-2006 (Funded by UN).

• WHO fellowship in Schistosomiasis Eradication and Control - Khartoum, Sudan, September 2001.

• WHO fellowship in Malaria and Planning its Control – WHO Regional Training Centre in Bandar Abbas, Iran September and November 2000.

Publications, fellowships, etc.:

Tabibi R, Consonni D, Brambilla G, Melzi d'Eril G, Sokooti M, Romanò L, Somaruga C, Vellere F, Varischi G, Colosi.(2012). ***Rischio di zoonosi per i lavoratori agricoli Lombardi*****. G Ital Med Lav Ergon. 34:3(2 Suppl):164-165.**

• Tabibi R, Corsini E, Brambilla G, Bonizzi L, Melzi d'Eril G, Rabozzi G, Sokooti M, Romanò L, Somaruga C, Vellere F, Zanetti A, Colosio C. (2012). ***Immune changes in animal breeders: a pilot study conducted in northern Italy.*** **Ann Agric Environ Med. 2012;19(2):221-5.**

• Colosio C, Somaruga C, Vellere F, Neri L, Rabozzi G, Romanó L, Tabibi R, Brambilla G, Baccalini R, d'Eril GV, Zanetti A, Colombi A. (2010). ***Biological risk prevention in agriculture and animal breeding: immunization strategies.*** **G Ital Med Lav Ergon;32(4 Suppl):302-5.**

• Giulia Rabozzi, Luigi Bonizzi, Eleonora Crespi, Chiara Somaruga, Maryam Sokooti,Ramin Tabibi, Francesca Vellere, Gabri Brambilla, Claudio Colosio. (2012). ***Emerging Zoonoses: the "One Health Approach*****".Saf Health Work ;3(1):77-83.**

• R. Tabibi, E. Corsini, C. Somaruga, M. Sokooti, G. Rabozzi, F. Vellere, C. Colosio,G. Brambilla. ***Occupational exposure to biohazards and endotoxins among agricultural workers in the region of Lombardy, Northern Italy*****. Toxicology Letters ; 205 August 28, 2011. p. S151.**

• **G. Rabozzi, C. Colosio, E. Crespi, C. Somaruga, M. Sokooti, R. Tabibi, F. Vellere, G. Brambilla (2011).** ***La dieta come elemento cardine della promozione della salute nei luoghi di lavoro nella ricerca scientifica e nella pratica. Primi risultati del progetto EU FAHRE*****. Giornale italiano di medicina del lavoro ed ergonomia;33:3, Suppl, 360-363.**

• **Keikhaei B, Zandian K, Ghasemi A, Tabibi R.. (2007)** ***Iron-deficiency anaemia among children in southwest Iran*****. Food Nutr Bull;28(4):406-11.**

• **Tabibi R., Farahnak A. (2002),** ***Ecology and Parasitic Infection of Caught Fish in Hoofel Lagoon and Its Relation to Public Health*****. Presented in the 10th International Congress of Parasitology, Vancouver, Canada.**

A Farahnak, 1I Mobedi, R Tabibi.(2002). ***Fish Anisakidae Helminthes in KHuzestan Province, South West of Iran.*** **Iranian Journal of Public Health 31 (3-4).**

Appendix V Health Surveillance Questaionnaire

U. O. C. di Medicina del Lavoro
-Centro Internazionale per la Salute Rurale-

CARTELLA SANITARIA E DI RISCHIO

Datore di lavoro
………………………………………………………………………………………..

Attività dell'Azienda pubblica o privata
…………………………………………………………………

Data di assunzione
..……………………………………………………………………………...

Tipo di assunzione – Contratto a tempo: ❒ Indeterminato ❒ Determinato ❒ Stagionale

Sede/i di lavoro………………………………………………………………………………………

Lavoratore ……………………………………………………….... sesso ❒ M ❒ F

Nato il ……………………… Luogo di nascita ……..…………………………………………...

Codice fiscale																

Domicilio (Comune e Prov.)
……………………………………………………………………………….

Via …………………………… Tel. …………………………………

Medico curante Dott.…………………………………………………………………….......................

Via …………………………………………………………… Tel. ………...………………………

La presente cartella sanitaria e di rischio è istituita per:

❒ prima istituzione ❒ esaurimento del documento precedente

❒ altri motivi ……..…………………………………………………………………….

Il Medico Competente ………………………………….

La presente cartella sanitaria e di rischio è costituita da n° ……………… pagine.

VISITA MEDICA PREVENTIVA

DATI OCCUPAZIONALI: i dati (*) sono forniti dal Datore di lavoro. (Indicare il n° degli allegati) ……….……

(*) **Destinazione lavorativa – Mansione** ……………………….…………………………………..………….

………………………………………………………………………………………

(*) F**attori di rischio cancerogeno:** ❐ NO ❐ SI

Indicare…...……………..……..………………………………..……………………………………… …………..

(*)F**attori di rischio professionale (descrivere e indicare livelli di esposizione) o profilo di esposizione per mansione.**

- **Rumore** ❐ NO ❐ SI Lex,8h = dB(A) ………… Lex,w = dB(A) …… ppeak= dB(C) …...……

Stima da profilo ……………………………………………………………………………………………

Indicare fonti di esposizione………..…………………………………………………………………………..

Valori attenuati da DPI ❐ NO ❐ SI ❐ Quali………………………………………………………..

- **Rischio chimico**: ❐ NO ❐ SI

Preparato o sostanza	Monitoraggio ambientale	Monitoraggio biologico	Rilevante/Irrilevante

- **Rischio biologico** ❐ NO ❐ SI

  Agenti: ………………….…………………………...……

Fonte: ❐ animali (allevamenti bovini, suini, ovi-caprini) ❐ ambiente ❐ manipolazione

- **Movimentazione manuale dei carichi:** ❐ NO

  ❐ SI indice NIOSH (se applicabile)…………………..………..

- **Movimenti ripetitivi arti superiori**:

  ❐ NO ❐ SI indice di rischio OCCRA (se applicabile)

- **Posture:** ❐ NO ❐ SI
  ………………………………………………………………………………..

- **Vibrazioni:** ❐ NO

  ❐ SI WBV (corpo intero) A(8) (…………….. m/s^2)
…………………………………………………….
Profilo per mansione……………………………………………………………………………………

  ❐ SI HAV (mano braccio) A(8) (…………….. m/s^2)
  ……………...……………………………………… Profilo per mansione
  ……………………………………………………………………………………….

- **Radiazioni non ionizzanti:** ❐ NO ❐ SI
  ………………..……………………….……………………….

  ❐ UV ❐ IR ❐ Lase …...........

- **Videoterminali:** ❐ NO ❐ SI ……….
  h/sett.……….….………..……….…………………

- **Lavoro notturno:** ❐ NO ❐ SI
  ………..…………………………………………………….…...

- **Lavoro a turni:** ❐ NO ❐ SI
  ………..…………………………………………………….…...

- **Lavori in quota / sospesi:** ❐ NO ❐ SI
  ……………..………………………………………………………

- **Mansioni tabellate a rischio alcol / sostanze psicoattive:** ❐ NO ❐ SI
  …..………………………...

- **Altri rischi:**…………………………………………………………………………………………
  ………

ANAMNESI LAVORATIVA:

Esposizioni precedenti: ❒ NO ❒ SI Anno di inizio lavoro ……….. Periodi di inattività (anni) ……….

Periodo:	Azienda:	Mansioni svolte:	Fattori di rischio:

Contemporanea esposizione presso altri datori di lavoro o attività professionale autonoma: ❒ NO ❒ SI
…………………………………………………………………………………………………
…………………………………………………………………………………………………
…………………………………………………………………………………………………
……………………………………….

Intolleranze all’uso di DPI nelle attività precedenti ❒ NO ❒ SI
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……………………………………………………………………………….……………………
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Prescrizioni limitative od inidoneità nelle attività precedenti ❒ NO ❒ SI
………………………………
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Altre notizie utili ai fini anamnestici lavorativi:
…………………………………………………………………
………………………………………………………………………………………………………
…………………………………………

ANAMNESI PERSONALE:

Infortuni e traumi: ❒ NO ❒ SI: Lavorativi ❒ SI: Extralavorativi

Data:	Tipo e sede della lesione:	Giorni di assenza:	Percentuale di invalidità:

Malattie professionali: ❒ NO ❒ SI

Data di denuncia:	Natura e causa:	Data di riconoscimento:	Percentuale di invalidità:

Invalidità extraprofessionali riconosciute: ❒ NO ❒ SI

Data di denuncia:	Invalidità Civile, assicuraz. private, INPS, altre….:	Natura e causa:	Percentuale di invalidità:

ANAMNESI FAMILIARE:

Patologie di rilievo in ascendenti e/o collaterali: ❒ NO ❒ SI

……….……

………………………………………………………………………………………………….………………

ANAMNESI FISIOLOGICA:

Alimentazione:
- ❒ Mista
- ❒ vegetariana
- ❒ Carnea
- ❒ antiallergica
- ❒ Altro(celiaca,etc.)

Alvo: ❒ regolare ❒ stipsi ❒ alterno ❒ diarroico ❒ altro:

Diuresi: ❒ regolare ❒ nicturia ❒ altro

Ritmo sonno/veglia: ❒ regolare ❒ insonnia ❒ ipersonnia ❒ altro:

Assunzione bevande alcoliche: ❒ NO ❒ SI:

❒ Vino:	unità alcoliche/ die:	dal:	al:
❒ Birra:	unità alcoliche/ die	dal:	al:
❒ Superalcolici:	unità alcoliche/ die	dal:	al:
❒ Altro:	unità alcoliche/ die	dal:	al:

Caffè: ❒ NO ❒ SI: Numero di tazze die:

The: ❒ NO ❒ SI: Numero di tazze die:

Fumatore: ❒ NO ❒ SI: ❒ EX

❒ Sigaretta:	numero die:	Dal	ha smesso il:
❒ Sigaro:	numero die:	Dal	ha smesso il:
❒ Pipa:	numero die:	Dal	ha smesso il:

Uso di Sostanze stupefacenti: ❒ NO ❒ SI:

❒ cannabinoidi:	❒ pregressa	❒ in atto
❒ eroina:	❒ pregressa	❒ in atto
❒ cocaina:	❒ pregressa	❒ in atto
❒ sintetiche:	❒ pregressa	❒ in atto

Assunzione abituale di farmaci: ❒ NO ❒ SI:

- ❒ Psicotropi: ❒ benzodiazepine ❒ antidepressivi ❒ antimaniacali ❒ antistaminici ❒ altro
- ❒ Nefrotossici:
- ❒ Ototossici:

❒ Epatotossici:
❒ Altri:

Attività sportive praticate: ❒ NO ❒ SI ❒ non agonistico tipo:
❒ agonistico: tipo:
❒ saltuario: tipo:

Patente di guida: ❒ NO ❒ SI: ❒ A ❒ B ❒ altre

Servizio Militare o civile: ❒ SI ❒ NO: ❒ Riformato: ❒ Esentato:

Scolarità .... Donatore di sangue ❒ SI ❒ NO

Hobbies:

Donne: Menarca: all'età di:
Cicli mestruali ❒ regolari ❒ irregolari
Menopausa: all'età di: ❒ fisiologica ❒ chirurgica ❒ farmacologica
Gravidanze: ❒ NO ❒ SI: n. ❒ a termine ❒ con taglio cesareo ❒ altro
Aborti spontanei: ❒ NO ❒ SI:

Vaccinazioni:

Tipo di vaccinazione:	Prima dose (data):	Seconda dose (data):	Terza dose (data):	Richiamo (data):	Titolo anticorpi (data):
Antitetanica					

ANAMNESI PATOLOGICA REMOTA

Allergie cutanee ❒ NO ❒ SI
…………………………………..……………………...……….…..
Allergie respiratorie ❒ NO ❒ SI
…………………………………..……..……………....……….……
Alt. otovestibolari ❒ NO ❒ SI
…………………………………………..……..…………………
Alterazioni umore ❒ NO ❒ SI
…………………………………………..………..………………
Artropatie ❒ NO ❒ SI
…………………………………………………………………

Broncopneumopatie ❒ NO ❒ SI
……………………………………………………………………………
Cardiopatie ❒ NO ❒ SI
……………………………………………………………………………
Dermopatie ❒ NO ❒ SI
……………………………………………………………………………
Diabete ❒ NO ❒ SI
……………………………………………………………………………
Dislipidemie ❒ NO ❒ SI
……………………………………………………………………………
Emopatie ❒ NO ❒ SI
……………………………………………………………………………
Epatopatie ❒ NO ❒ SI
……………………………………………………………………………
Ernie addominali ❒ NO ❒ SI
……………………………………………………………………………
Ipertensione arteriosa ❒ NO ❒ SI
……………………………………………………………………………
Ipoacusia i ❒ NO ❒ SI
……………………………………………………………………………
Nefropatie ❒ NO ❒ SI
……………………………………………………………………………
Neuropatie ❒ NO ❒ SI
……………………………………………………………………………
Pat. Gastrointestinali ❒ NO ❒ SI
……………………………………………………………………………
Psicopatia ❒ NO ❒ SI
……………………………………………………………………………
Tireopatie ❒ NO ❒ SI
……………………………………………………………………………

Blocchi rachidei acuti nell'ultimo anno ❒ NO ❒ SI
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Lombosciatalgie ❒ Dx ❒ Sx ❒ NO ❒ SI
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Cervicobrachialgie ❒ Dx ❒ Sx ❒ NO ❒ SI
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Fratture pregresse ❒ NO ❒ SI
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Interventi chirurgici ❒ NO ❒ SI
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Note:
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Eventuali schede per rischi specifici, in allegato:

❒ Valutazione del rachide	❒ Questionario patologie neoplastiche nasali
❒ Valutazione arti superiori	❒ Valutazione rischio cardiovascolare
❒ Lavoro a videoterminale	❒ Questionario sostanze dermolesive
❒ Lavoro in quota e sospeso	❒ Schede per il "mobile worker"
❒ Lavoro notturno	❒ Valutazione rischio dipendenze
❒ Questionario patologie respiratore	❒

ANAMNESI PATOLOGICA PROSSIMA E STATO ATTUALE
……………………………………………………………………………………………………
…………………………………………………………………………………..……………
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Il Sottoscritto dichiara che quanto riferito al Medico e, quindi riportato nell'anamnesi, corrisponde al vero.
Il Sottoscritto si impegna, inoltre, ad informarlo in futuro, su ogni variazione del proprio stato di salute.

Data ………. …………. **Per presa visione, il Lavoratore**
…………………………..………………………….

ESAME OBIETTIVO GENERALE

Condizioni generali: Buone ❒ SI ❒ NO
……………………………………………………………
Peso Kg. …….. Altezza cm. ….…. BMI $(Kg/m)^2$ ……… Trofismo muscolare
……………………………

Collo e tiroide: Nella norma ❒ SI
❒ NO
……………………………………………………………………………………………………..…

Cute ed annessi: Cute trofica ed elastica; annessi secondo sesso ed età ❒ SI
❒ NO
…..…………………………………………………………………………………………………

App. cardiovascolare: F. C. ………… P.A. …………………..
Obiettività cardiologica normale ❒ SI
❒ NO ………………………………………………………………………………………………

Alterazioni dei polsi periferici, edemi, varici venose ❒ NO
❒ SI
………

Apparato respiratorio: Torace simmetrico, normoespansibile, normofonesi plessica, FVT e MV nella norma
❒ SI
❒ NO
……………………………………………………………………………………………..………
………
………

Addome: Piano, trattabile, indolente alla palpazione superficiale e profonda ❒ SI
Organi ipocondriaci nei limiti di norma ❒ SI
❒ NO
…………………………………………………………………………………………..………
………
………

Rachide: Nella norma ❒ SI ❒ NO
Dolorabilità alla digitopressione, alla flesso/estensione e rotazione nei tratti valutati
❒ SI
……
………
………
❒ NO

Arti superiori: Nella norma ❒ SI
❒ NO
…………………………………………………………………………………………..………
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………

Arti inferiori: Nella norma ❒ SI ❒ NO
……………………………………………………………………………………………………..………
………
………

Sistema nervoso: Obiettività nella norma ❒ SI
❒ NO
……………………………………………………………………………………………………...………
………
………

Occhi / vista: Nella norma, globi oculari in asse, pupille isocoriche, normoreagenti ❒ SI ❒ NO
…………………………………………………………………………………………………...………

OSSERVAZIONI e/o CONSIGLI:
………
………
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Accertamenti integrativi specialistici e/o di laboratorio (tipo di accertamento e n° di riferimento dei referti allegati):

……………………………………………………………………………………………
……………………………………………………………………………………………
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Valutazioni conclusive dei dati clinico-anamnestici e dei risultati degli accertamenti integrativi, in relazione ai rischi occupazionali:
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
…………………………………………………………

Giudizio di idoneità alla mansione:

Luogo e data:

Il Medico
Competente
(Timbro e firma)

VISITA MEDICA PERIODICA N° ………….. in data ………………………

Lavoratore ………………………………………………………….... sesso ❒ M ❒ F

Motivazione: ❒ idoneità alla mansione
……………………….……………………………………………
❒ cambio mansione
…………………………..…………………………………………………….
❒ su richiesta
……………….……………….…………….………….………………………….
❒ fine rapporto di lavoro
……………………..………………………………..…………………
❒ ………………………………………..
….…………………………………………….......

DATI OCCUPAZIONALI: i dati (*) sono forniti dal Datore di lavoro, in caso di variazione rispetto all’ultima visita medica. (Indicare il n° degli allegati)

Variazione destinazione lavorativa/mansione: NO ❒ SI ❒
……..…...…………………………………………………

(*) **Destinazione lavorativa – Mansione**
……………………….……………...………………………..………….
……………………………………………………………….…………………………………………………
………………

Indicare le eventuali variazione a quanto segnalato in precedenza:

ANAMNESI INTERCORRENTE:
……
……
……
……
…………………………………………………………………

E’ o è stato esposto a pesticidi?

Quali prodotti?...

Su quali colture ha applicato?..

Con che frequenza stagionale?...

Qual era/é l'estensione dell'area trattata?

………………………………………………………………… Modalità di applicazione e

attrezzature: ………………………………………………………………….

Utilizzava
DPI?...

Ha lavorato presso allevamenti?...

Infortuni e traumi: ❒ NO ❒ SI: Lavorativi ❒ SI: Extralavorativi

Data:	Tipo e sede della lesione:	Giorni di assenza:	Percentuale di invalidità:

Malattie professionali: ❒ NO ❒ SI

Data di denuncia:	Natura e causa:	Data di riconoscimento:	Percentuale di invalidità:

Invalidità extraprofessionali riconosciute: ❒ NO ❒ SI

Data di denuncia:	Invalidità Civile, assicuraz. private, INPS, altre….:	Natura e causa:	Percentuale di invalidità:

Contemporanea esposizione presso altri datori di lavoro o attività professionale autonoma:

❒ NO ❒ SI (indicare gli agenti)
……………………………………………………………………………………………………
……………………………………………………………………………………………………
…………………………

Intolleranze all'uso di DPI nelle attività precedenti ❒ NO ❒ SI

……………………………………………………………………………………………………
……………

Eventuali schede per rischi specifici, in allegato:

❒ Valutazione del rachide	❒ Questionario patologie neoplastiche nasali
❒ Valutazione arti superiori	❒ Valutazione rischio cardiovascolare
❒ Lavoro a videoterminale	❒ Questionario sostanze dermolesive
❒ Lavoro in quota e sospeso	❒ Schede per il "mobile worker"
❒ Lavoro notturno	❒ Valutazione rischio dipendenze
❒ Questionario patologie respiratore	❒

Osservazioni:
……………………………………………………………………………………………………
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…………………………………………………………………

Il Sottoscritto dichiara che quanto riferito al Medico e, quindi riportato nell'anamnesi, corrisponde al vero.
Il Sottoscritto si impegna, inoltre, ad informarlo in futuro, su ogni variazione del proprio stato di salute.

Data ………. …………….. **Per presa visione, il Lavoratore**
……………………………………………………………

EDVENTUALI VARIAZIONI AL PROGRAMMA DI SORVEGLIANZA SANITARIA E MOTIVO:

ESAME OBIETTIVO GENERALE

Condizioni generali: Buone ❒ SI ❒ NO
…………………………………………………………
Peso Kg. …….. Altezza cm. ….…. BMI $(Kg/m)^2$ ……… Trofismo muscolare
……………………………

Collo e tiroide: Nella norma ❒ SI
❒ NO
……………………………………………………………………………………………………..…

Cute ed annessi: Cute trofica ed elastica; annessi secondo sesso ed età ❒ SI
❒ NO
…..……………………………………………………………………………………………………

App. cardiovascolare: F. C. ………… P.A. …………………..
Obiettività cardiologica normale ❒ SI
❒ NO ……………………………………………………………………………………………

Alterazioni dei polsi periferici, edemi, varici venose ❒ NO
❒ SI
………

Apparato respiratorio: Torace simmetrico, normoespansibile, normofonesi plessica, FVT e MV nella norma
❒ SI
❒ NO
……………………………………………………………………………………………..………
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Addome: Piano, trattabile, indolente alla palpazione superficiale e profonda ❒ SI
Organi ipocondriaci nei limiti di norma ❒ SI
❒ NO
…………………………………………………………………………………………..………
………
………

Rachide: Nella norma ❒ SI ❒ NO
Dolorabilità alla digitopressione, alla flesso/estensione e rotazione nei tratti valutati
❒ SI
……
………
………
❒ NO

Arti superiori: Nella norma ❒ SI
❒ NO
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Arti inferiori: Nella norma ❒ SI ❒ NO
……………………………………………………………………………………………..………
………

Sistema nervoso: Obiettività nella norma ❒ SI
❒ NO
……………………………………………………………………………………………..………
………

Occhi / vista: Nella norma, globi oculari in asse, pupille isocoriche, normoreagenti ❒ SI
❒ NO
……………………………………………………………………………………………..………

OSSERVAZIONI e/o CONSIGLI:
………

Accertamenti integrativi specialistici e/o di laboratorio (tipo di accertamento e n° di riferimento dei referti allegati):

…………………………………………………………………………………………………
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Valutazioni conclusive dei dati clinico-anamnestici e dei risultati degli accertamenti integrativi, in relazione ai rischi occupazionali:

………………………………………………………………………………………
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Giudizio di idoneità alla mansione:

Luogo e data:

Il Medico
Competente
(Timbro e firma)

APPENDIX VI PUBLICATIONS

ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 2, 221-225
www.aaem.pl

Immune changes in animal breeders: a pilot study conducted in northern Italy

Ramin Tabibi[1], Emanuela Corsini[2], Gabri Brambilla[1], Luigi Bonizzi[3], Gianlodovico Melzi d'Eril[4], Giulia Rabozzi[1], Maryam Sokooti[1], Luisa Romanò[5], Chiara Somaruga[1], Francesca Vellere[1], Alessandro Zanetti[5], Claudio Colosio[1]

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[2] *Laboratory of Toxicology, Department of Pharmacological Sciences, University of Milan, Italy*
[3] *Department of Animal Pathology, Hygiene and Veterinary Public Health, University of Milan, Italy*
[4] *Department of Medicine, Surgery and Dentistry, University of Milan, Italy*
[5] *Department of Public Health – Microbiology – Virology, University of Milan, Italy*

Tabibi R, Corsini E, Brambilla G, Bonizzi L, Melzi d'Eril G, Rabozzi G, Sokooti M, Romanò L, Somaruga C, Vellere F, Zanetti A, Colosio C. Immune changes in animal breeders: a pilot study conducted in northern Italy. Ann Agric Environ Med. 2012; 19(2): 221-225.

Abstract

Objective: Farming is associated with exposure to a wide variety of risk factors including organic dusts, endotoxins, allergens and other chemicals. The ability of some of these agents to interact with the immune system is demonstrated in the presented study which was undertaken to evaluate the relationship between pig and cow breeding, and the immune system early changes. Particular attention is paid to selected serum cytokines.

Methods: Sixty four animal breeders (36 cattle and 28 pig breeders) were selected as the exposed group, and 32 rural workers not engaged in animal breeding were utilised as the controls. Personal data were collected through a questionnaire, and selected serum parameters measured, including cytokines IL-6, IL-8, IL-10, IFNγ and TNFα, immunoglobulins and proteins, and total and differential white blood cell counts.

Results: The study stresses the significant increase of TNF-α, IL-8, and IL-10 in animal breeders, with the highest values in pig breeders, and a slight but statistically significant increase in albumin and total serum proteins.

Conclusions: The findings of the presented study suggest a condition of immune system activation in animal breeders, with the highest levels observed in pig breeders. These changes may be attributable to exposure to organic dusts, endotoxins, or to the different biological agents present in the rural environment. The prognostic significance of these findings, however, remains unclear, but the observed changes might be indicative of a risk of developing respiratory toxic and allergic diseases, which need to be further investigated.

Key words

pig breeders, farmers, inflammatory cytokines, interleukins, biological risks, organic dust, endotoxins

INTRODUCTION

Animal breeders are occupationally exposed to numerous agents that are potentially capable of interacting with the immune system. Some of these agents include bacteria such as *Brucella* spp., *Erysipelothrix rhusiopathiae*, *Leptospirosis* spp., *Mycobacterium* spp., *Streptococcus* spp., as well as viruses, such as *Hepatitis E and Influenza* [1, 2, 3, 4, 5, 6, 7, 8, 9]. The contact with these biological agents exposes workers to a risk that is still poorly defined.

In addition to microorganisms, occupational exposure on animal breeding farms might also result in contact with other biological risk factors, in particular organic dusts. In general, the air in livestock buildings contains a large variety of microorganisms, gases, and a considerable amount of dusts which can remain suspended in the air for long periods, and can therefore be inhaled [10, 11]. Dusts are characterized by their heterogeneous composition, containing endotoxin, lectins, pollens, feeds, and antibiotic residues. There is strong epidemiological evidence that organic dusts and bacteria can cause infectious and allergic diseases, both in animals and farm workers [12]. High exposure to organic dusts, especially when contaminated with large amounts of endotoxin, may result in a flu-like disease, with symptoms such as fever, chills, dry cough, malaise, mild dyspnea, headache and muscle pain, the so-called 'organic dust toxic syndrome' (ODTS) [13, 14]. In subjects without any sign of overt diseases, inhalation of organic dusts might also results in slight changes, indicative of an immune system activation such as increase in blood cell count, IL6 and TNFα observed particularly in winter when ventilation is reduced [16, 17]. It is unclear whether these changes represent an early adverse effect, able to evolve into overt diseases, or only adaptive and transient changes consequent with exposure to immune system activators.

Studies addressing the interactions between the immune system and rural indoor environment, in particular animal breeding farms, are few and far between, and no firm conclusions have been drawn from them [18, 19]. The presented pilot study was carried out with the aim of studying selected immune parameters in a group of animal breeders

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Commentary

Emerging Zoonoses: the "One Health Approach"

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Zoonoses represent a public health risk recently pointed out by the spreading of previously unknown human infectious diseases emerging from animal reservoirs such as severe acute respiratory syndrome and avian influenza caused by *H5N1*-virus. These outbreaks have shown that animal breeding activities can pose a significant public health risk. Until now, the risk of zoonoses has probably been underestimated, particularly in occupational settings. The emergence or re-emergence of bacterial (*Mycobacterium bovis and Brucella spp*) or viral (*hepatitis E* virus) infections shows that zoonoses should be considered as emerging risks in agricultural and animal breeding and should be addressed by specific preventive interventions. Close cooperation and interaction between veterinarians, occupational health physicians and public health operators is necessary, for a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment. This is what the One Health Approach was intended to be.

Key Words: Biological risk, Agriculture, Zoonoses, Emerging and reemerging zoonoses

According to the World Health Organization (WHO, http://www.who.int/topics/zoonoses/en/), a zoonoses can be defined as "any disease or infection caused by all types of agents (bacteria, parasites, fungi, viruses and unconventional agents) transmissible from vertebrate animals to humans and *vice-versa*". During recent decades, the public health risk represented by zoonoses was suggested by the onset of outbreaks and epidemics of previously unknown human infectious diseases that emerged from animal reservoirs such as Ebola virus, West Nile virus, Nipah virus, Hanta virus, Creutzfeldt-Jakob disease.

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More recently, severe acute respiratory syndrome (SARS), highly pathogenic avian influenza (HPAI) viruses [1] have shown that biological agents and animal breeding activities can pose a significant public health risk, because several animal infectious diseases are not only endemic but also epidemic-prone, such as leptospirosis, brucellosis and rabies [2]. Therefore, these agents can potentially cause epidemics at any time. In this light, we can affirm that the risk of zoonoses, particularly in occupational settings, has been probably underestimated in past years. This has been highlighted by epidemics that originated from the animal breeding sector, and, in some cases, from specific and identified animal breeding and feeding modalities.

The example of HPAI clearly shows that any emerging disease may rapidly, for several reasons, become endemic, causing a public health concern. Therefore, emerging and re-emerging diseases represent priorities for prevention and the creation of an early warning system that is specifically targeted

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AG 06

RISCHIO DI ZOONOSI PER I LAVORATORI AGRICOLI LOMBARDI

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RIASSUNTO. Questo lavoro descrive uno studio trasversale condotto, in Lombardia, nel periodo 2010-2011, su 96 lavoratori del settore agricolo. Di questi, 28 erano allevatori suinicoli, 36 di bovini e 32 agricoltori, non in contatto con animali. Sono state raccolte, attraverso questionari, informazioni cliniche e socio-demografiche e sono stati prelevati, ad ognuno, 10 ml di sangue venoso, per la determinazione di anticorpi anti zoonosi. I risultati mostrano una più alta percentuale di anticorpi anti *Coxiella* e *Leptospira* negli allevatori (51.6% e 59.4% rispettivamente) rispetto agli agricoltori (28.1% e 43.7%), suggerendo, quindi un possibile rischio di zoonosi negli allevamenti, che appare attualmente sottovalutato.

ZOONOTIC RISK FOR AGRICULTURAL WORKERS IN LOMBARDY REGION

ABSTRACT. *This cross-sectional study was conducted in 2010-2011, in Lombardy region to investigate the risk of zoonoses in farmers and animal breeders. Ninety-six workers were randomly selected including 28 pig-breeder, 36 cattle-breeder and 32 farmers not in contact with animals. Socio-demographic and clinical information were collected through questionnaires and 10 ml of venous blood were taken for determination of antibodies against zoonoses. The results showed a higher percentage of Coxiella and Leptospira antibodies in breeders (51.6% and 59.4% respectively) compared to farmers (28.1% and 43.7%), suggesting of possible risk of zoonoses in livestock and the fact that zoonoses are probably* underestimated.

Key words: *Zoonoses, Agricultural workers, Region of Lombardy (Italy)*

INTRODUZIONE

Nell'agricoltura e nell'allevamento i lavoratori sono frequentemente esposti ad agenti biologici potenzialmente nocivi, e il rischio di zoonosi (patologie trasmesse dagli animali all'uomo) si dimostra sempre più importante: in particolare, circa il 75% delle patologie infettive emergenti, che hanno colpito la specie umana negli ultimi 10 anni, avevano come agenti eziologici i patogeni provenienti da animali o da derivati animali (1, 2). In alcuni casi l'epidemia è stata causata dal contatto diretto con animali selvatici infetti (come nel caso del virus West Nile) o dal contagio di animali domestici o di allevamento da parte di animali selvatici infetti, che a loro volta hanno infetatto l'uomo (come nel caso della trasmissione dei virus influenzali aviari a suini ed umani). Le zoonosi hanno potenzialmente un impatto significativo sulla salute dei lavoratori e possono raggiungere anche la popolazione generale attraverso il consumo di alimenti di origine animale contaminati.

Il Centro Internazionale per la Salute Rurale dell'Azienda Ospedaliera San Paolo di Milano, Centro di Collaborazione dell'Organizzazione Mondiale della Sanità per la Medicina del Lavoro ha recentemente condotto uno studio pilota per verificare se negli allevatori della Regione Lombardia vi sia un rischio significativo di trasmissione di alcune specifiche zoonoosi dagli animali da reddito (bovini, suini) all'uomo e verificare la necessità di eventuali approfondimenti. Tale studio è stato condotto indagando la sieroprevalenza di anticorpi verso alcuni specifici patogeni (*Brucella, Salmonella, Coxiella* e *Borrellia*), negli allevatori e utilizzando quale popolazione di controllo agricoltori non coinvolti in attività di allevamento. I patogeni sono stati selezionati in base alla rilevanza potenziale del rischio occupazionale e all'esistenza di diverse tipologie di contatto (diretto e mediato da vettori).

MATERIALI E METODI

Questo studio pilota, di tipo trasversale, è stato condotto, nel periodo 2010-2011, su lavoratori di aziende agricole lombarde di piccole dimensioni, cui il nostro Centro già da anni fornisce la sorveglianza sanitaria di legge sui luoghi di lavoro.

Per questo specifico studio sono stati selezionati 96 lavoratori, di cui 36 allevatori di bovini 28 allevatori di suini e, quale gruppo di controllo, 32 agricoltori non coinvolti in attività di allevamento.

Tutti i lavoratori partecipanti al progetto sono stati adeguatamente informati circa gli obiettivi e le modalità di svolgimento dello studio, precedentemente approvato dal comitato etico dell'Azienda Ospedaliera San Paolo e condotto nell'ambito delle attività routinarie di sorveglianza sanitaria sui luoghi di lavoro.

Le informazioni cliniche e socio-demografiche necessarie per l'individuazione delle caratteristiche generali della casistica allo studio e per l'evidenziazione di possibili fattori di confondimento sono state ottenute dalle cartelle cliniche dei soggetti, conservate come previsto dalla legge presso il nostro Centro. Nella raccolta dei dati è stato garantito il rispetto dell'anonimato dei partecipanti e la protezione di eventuali dati sensibili.

La ricerca degli anticorpi anti *Coxiella burnetii*, anti *Leptospira spp*, anti *Brucella spp*, anti *Borrelia burgdorferi* e anti *Salmonella spp*. sono state effettuate con metodi immuno-enzimatici (*v.* Tabella I) su campioni di siero ottenuti da un prelievo di sangue venoso (10 mL) effettuato al momento della visita periodica annuale a questi lavoratori. I campioni di sangue sono stati processati entro 4 ore dalla raccolta ed il siero ottenuto è stato congelato ad una temperatura di -20 °C, fino al momento delle analisi. Le analisi sono state condotte con l'uso di normali kit commerciali di laboratorio, mediante tecniche immunoenzimatiche ELISA. Sono stati utilizzati il test chi quadrato e la regressione logistica per valutare a prevalenza di sieropositività ai singoli agenti patogeni nei diversi gruppi. L'analisi statistica è stata realizzata con il software SPSS versione 18 (SPSS Inc., Chicago, IL, USA).

RISULTATI

Lo studio ha coinvolto 96 lavoratori agricoli (91 maschi, 5 femmine) con un'età compresa tra i 19 ed i 70 anni (mediana 42 anni). La maggioranza dei lavoratori era italiana o proveniente dall'Unione Europea (70/96, 73%) ed i restanti erano originari di paesi extraeuropei.

La Tabella I riassume i risultati delle analisi condotte sui campioni di sangue venoso ottenuti dai lavoratori agricoli. Nessuno dei soggetti si è dimostrato sieropositivo nei confronti di *Brucella* e *Salmonella* e in generale non è emersa una differenza significativa nella prevalenza di sieropositività agli agenti patogeni tra allevatori e agricoltori non-allevatori.

Al contrario, la prevalenza di sieropositività verso *Coxiella* era quasi il doppio negli allevatori rispetto ai soggetti di controllo ($p = 0.09$), mentre per quelle verso *Leptospira* e *Borrelia* non si evidenziavano differenze di rilievo.

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Occupational exposure to biohazards and endotoxins among agricultural workers in the region of Lombardy, Northern Italy

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Purpose: Farming is associated with a wide variety of hazardous exposures including physical, chemicals and biological agents. Animal farmers are exposed to dust, which contains microorganisms, mycotoxins, endotoxins, animal feed particles, allergens and chemical agents. Organic dust exposure is known to cause allergic and non-allergic rhinitis, and organic dust toxic syndrome. Measurements of serum cytokine levels have been performed as biomarkers and strong predictors of diseases in many epidemiologic studies. This study was undertaken to evaluate the relationship between occupational health hazards and serum pro-inflammatory cytokines among agricultural workers in north of Italy.

Methods: A pilot study was conducted in 2010 and 100 subjects working in agricultural enterprises in the region of Lombardy were enrolled into the study. Serum cytokines including interleukin IL-6, IL-8, IL-10, IFN gamma and tumor necrosis factor alpha were measured. Cytokines were assessed by commercially available enzyme-linked-immunosorbent assay (ELISA).

Results of the study: Compared to control subjects, increased TNF-alpha, IL-8 and IL-10 levels were found in animal breeders, with a statistically significant relationship between type of job (breeder/non breeder workers) and increased serum pro-inflammatory cytokines. Results suggest that animal breeders might be at higher risk of biological hazards than other farmers with less contact to animals. Blood cytokine assay may also be useful to identify individual responsiveness to endotoxins at work place. The relationship between exposure to organic dust, microorganisms, endotoxins and other chemicals in the work place and disease needs further research.

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La dieta come elemento cardine della promozione della salute nei luoghi di lavoro nella ricerca scientifica e nella pratica. Primi risultati del progetto EU FAHRE

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RIASSUNTO. La globalizzazione dei mercati ha portato a rapidi cambiamenti nelle abitudini alimentari e negli stili di vita nei paesi sviluppati aumentandone gli standard di vita, la disponibilità e la varietà di cibo. Questi elementi, combinati con abitudini alimentari errate e ridotta attività fisica hanno portato ad un incremento di malattie croniche (obesità e diabete mellito), malattie cardiovascolari e alcune neoplasie. Nasce quindi la necessità di sviluppare attività di promozione della salute per le quali i lavoratori rappresentano un gruppo ideale poiché sono reperibili in numero significativo, nello stesso luogo e in orari prestabiliti e sono seguiti da un Medico Competente che ne conosce lo stato di salute e può individuare le più appropriate strategie per lo sviluppo di tali attività. In questo scenario rinnovato interesse deve essere posto al rapporto tra dieta e salute. Per questo il nostro gruppo sta partecipando al progetto FAHRE (Food and Health Research in Europe), che ha lo scopo di stabilire lo stato dell'arte della ricerca riguardante alimentazione e salute identificandone i punti di forza ed i punti deboli proponendo strategie migliorative a livello di coordinazione, comunicazione, regolamentazione innovazione.

ABSTRACT. Diet as a key element of health promotion at the workplace in scientific research and practice. First results of the EU project FAHRE. The globalization of markets have led to rapid changes in diet and lifestyle in the developed countries, where living standards have improved, and availability and variety of food has increased while physical activity decreases have led to an augment in chronic diseases (obesity and diabetes mellitus), cardiovascular diseases and some specific cancers. In this context there is a need to develop health promotion activities. Workers represent a very appropriate group for such activities because they are relatively homogeneous, easily reachable because they can be found together in the same place and time and the occupational physician has good knowledge of the of health status of individual worker. In this scenario, a renew interest must be posed to the relationship between diet and health. In this context our Group is participating at the FAHRE project (Food and Health Research in Europe). The project aims to establish the state of the art of research at the interface of nutrition and health in the European Union, identifying its strengths and weaknesses in order to propose strategies to increase coordination and improve its functioning as a European Research Area.

Key words: food and health, health promotion, FAHRE project.

Introduzione

Nel corso dell'ultimo decennio, l'industrializzazione, l'urbanizzazione, lo sviluppo economico e la globalizzazione dei mercati hanno portato a rapidi cambiamenti nelle abitudini alimentari e negli stili di vita nei paesi cosiddetti sviluppati, nei quali gli standard di vita sono migliorati e la disponibilità e la varietà di cibo sono aumentate. Questi elementi, sommati alla ridotta attività fisica, all'aumentato consumo di tabacco, ad abitudini alimentari errate come diete altamente energetiche ad alto contenuto di grassi e povere di carboidrati non raffinati hanno portato ad un significativo incremento di malattie croniche quali obesità, aterosclerosi e diabete mellito. Importante ricordare il significativo "burden of disease" di tali patologie, tra le quali il solo diabete causa attualmente 4 milioni di decessi/anno nel mondo (9% del totale). L'obesità nei paesi sviluppati porta ad un elevatissimo costo in termini di riduzione della produttività, assenteismo, riduzione della qualità della vita e significativo incremento di morbilità (Wändell PE, 2008).

Negli ultimi anni la ricerca scientifica ha peraltro identificato una nuova condizione patologica, definita "sindrome metabolica" che secondo i criteri definiti nel 2001 dal "National Cholesterol Education Program's Adult Treatment Panel III report (ATP III)" e secondo l'American Hearth Associations è caratterizzata dall'associazione di tre o più tra le seguenti condizioni: obesità centrale (circonferenza vita maggiore di 102 cm per gli uomini e 88 cm per le donne), ipertrigliceridemia (maggiore di 150 mg/dl o utilizzo di farmaci allo scopo di ridurla), basso valore di colesterolo HDL plasmatico (minore di 40 mg/dl per gli uomini e 40 mg/dl per le donne o uso di farmaci al fine di aumentarne il valore), ipertensione (o utilizzo di farmaci antiipertensivi) e glicemia a digiuno maggiore di 100 mg/dl (o uso di farmaci ipoglicemizzanti). I soggetti che soddisfano tali criteri presentano un più alto rischio di eventi cardiovascolari e di diabete insulino indipendente (di tipo 2). Nel 2008, tra i 57 milioni totali di decessi avvenuti nel mondo, 63% (36 milioni) è stato causato principalmente da malattie cardiovascolari, diabete cancro e malattie respiratorie croniche (Alwan A. *et al.*, 2010). Inoltre, l'80% dei decessi per questo tipo di malattie è registrato nei paesi a medio-basso reddito.

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Strategie vaccinali per la prevenzione del rischio biologico in agricoltura e zootecnia

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RIASSUNTO. Le patologie prevenibili con vaccino sono tuttora un obiettivo dei programmi di salute pubblica in Italia e nel mondo. Malattie per cui i programmi vaccinali sono ampiamente consolidati continuano a costituire un problema di salute pubblica, basti pensare ai casi di difterite notificati nel 2009 in alcuni Paesi UE o ai 64 casi di Tetano notificati in Italia nel 2006. L'agricoltura e l'allevamento sono settori a forte rischio biologico, in parte per le caratteristiche dell'ambiente di lavoro e in parte per la tipologia di infortuni in cui incorrono i lavoratori. Inoltre, nel comparto sono presenti due sottogruppi maggiormente esposti a rischio: gli ultrasessantaquattrenni e i migranti, generalmente non coperti, ad esempio, nei confronti del tetano. Tra le patologie infettive più tipiche del comparto, solo il tetano è efficacemente controllabile con la vaccinazione degli addetti. Per leptospirosi, salmonellosi ed epatite E, hanno invece maggiore rilevanza programmi di informazione e formazione sui rischi per la salute e sui comportamenti da seguire per evitarne il contatto. Tali programmi devono tener conto delle barriere linguistiche e culturali.

Parole chiave: zoonosi occupazionali, rischio biologico, vaccinazione.

ABSTRACT. *BIOLOGICAL RISK PREVENTION IN AGRICULTURE AND ANIMAL BREEDING: IMMUNIZATION STRATEGIES.* ***Vaccine preventable diseases are, so far, a main focus of Public Health programmes all over the world since people still die in consequence of Dyphteria or Tetanus. Biological risk is widely represented in agriculture and animal breeding, due to environmental characteristics and to injury typology. Moreover, aged people and migrants represent a significant part of the workforce. These two groups are, for instance, more exposed to Clostridium tetani infection because not fully immunized. Among infectious diseases that can affect agricultural workers, just tetanus can be well controlled by immunization programmes. Teaching and training activities are the most important tools to get protection against Leptospira interrogans, Salmonella spp and hepatitis E Virus infection. As for every training activity, linguistic and cultural barriers have to be taken into account.***

Key words: *occupational zoonoses, biological risk, vaccination.*

Introduzione

Le malattie prevenibili con vaccino sono ancora una priorità per le strategie di salute pubblica non solo nei paesi in via di sviluppo. Si considerino i casi di difterite notificati nel 2009 in alcuni Paesi dell'Europa a 27: 1 caso in Svezia, 6 in Lettonia, 4 in Germania (WHO 2010). L'Organizzazione Mondiale della Sanità riporta inoltre dati relativi all'incidenza di un'altra patologia controllabile con un vaccino sicuro, il tetano. Nel 2005 sono stati notificati 15,516 casi nel mondo e tra 2000 e il 2003 sono stati 290 i morti per questa patologia. In Italia nel 2006 ci sono stati 64 casi, con un'incidenza del tetano circa 10 volte superiore alla media degli altri paesi europei (1). L'andamento della diffusione delle malattie infettive prevenibili con vaccino ha inoltre recentemente assunto nuove caratteristiche, in relazione al fenomeno migratorio (2).

Le patologie prevenibili con vaccino in agricoltura

L'agricoltura e l'allevamento sono comparti ad importante rischio biologico, sia per il frequente contatto con materiale potenzialmente contaminato, sia per l'alto indice infortunistico che rende, per esempio, il rischio tetano particolarmente significativo.

Di seguito si intendono presentare le patologie infettive prevenibili con vaccino più rilevanti per il comparto agro-zootecnico e discutere l'applicabilità e il valore preventivo dei programmi di immunizzazione. Verrà analizzato in dettaglio il caso del tetano e verranno quindi discusse brevemente le seguenti patologie potenzialmente rilevanti nel comparto: salmonellosi, leptospirosi ed epatite E.

Tetano

Il tetano è una patologia infettiva acuta sostenuta da una esotossina prodotta nell'organismo umano durante la proliferazione del clostridium tetani, batterio sporigeno anaerobio, gram positivo, ampiamente distribuito nel terreno, in cui le spore possono rimanere vitali per anni, nella polvere e nelle feci degli erbivori. Il tetano può insorgere