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ORIGINAL ARTICLE

Occupational exposure to zoonotic agents among agricultural workers in Lombardy Region, northern Italy

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Abstract

Objectives: This study was conducted in Northern Italy with the aim of defining the risk of agricultural workers' contact with biological agents through the determination of serum antibodies against selected zoonotic agents. Immunity against tetanus was also investigated.
Methods: Two groups of agricultural workers consisting of 153 animal breeders (exposed) and 46 non- breeders (controls) were included in the study. In a first group of 103 workers (89 exposed and 14 controls) the serum concentrations of antibodies against *Hepatitis E Virus* (HEV) were measured, whereas in the second group of 96 workers (64 exposed and 32 controls) the serum concentrations of antibodies against *Leptospira spp.*, *Coxiella burnetii*, *Borrelia burgdorferi*, *Brucella spp.* and *Salmonella spp.* were addressed. Imunization against tretanus was also studied in this group.
Results: Animal breeders showed higher rates of IgG antibodies against *Coxiella burnetii* (50% vs. 31.2%), and *Leptospira spp.* (59.4% vs. 43.7%). Results of logistic regression analysis revealed that breeder workers showed a tendency to have higher prevalence of positivity for antibodies to *Leptospira spp.*and *Coxiella burnetii* than non-breeders (ORs ~ 3). Only one exposed subject showed antibodies against hepatitis E (none in controls), but when tested with another commercially available kit the percentage of anti HEV IgG positive subjects increased to 22.3% in the exposed, while none of the controls showed positive. None of the subjects showed antibodies against *Salmonella spp.* and *Brucella spp.* Italians and other European workers have better protection against tetanus (91%) compared to non-EU workers (81%).
Conclusions: The higher frequency of the presence of serum antibodies to zoonotic agents (*e.g. Leptospira spp.* and *Coxiella burnetii*) in animal breeders suggests that they are more exposed to biological agents than workers not involved in animal breeding activities. The risk of contact with HEV deserves further studies because the adoption of different assays can result in significantly different results. The promotion of immunization of agricultural workers might be a priority, in particular for migrants.

Key words
biological risks, zoonoses, farmers, animal breeders, Lombardy region, antibody

INTRODUCTION

It is well known that animals which are bred for commercial and non-commercial purposes might develop transmissible diseases which can be transmitted to other animals and, in some cases, to humans [1]. According to the WHO (1959), infections and diseases naturally transmitted between vertebrate animals and humans are defined as 'zoonoses' [2]. Zoonoses can be associated with the handling of infected wild or domestic animals or their products, might have a significant impact on human health, and contribute to the overall burden of infectious diseases [3]. Evidence suggests that 75% of emerging pathogens and 61% of all infectious organisms are zoonotic [4]. Important recent examples of zoonoses include the emergence of the 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 [5, 6]. 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 Cozzio (2003), biological agents accompany nearly 60% of farm activities, and farmers are generally not aware of their presence [7].

There is growing evidence that animal husbandry implies the presence of biohazards for both producers and consumers. The recent outbreaks of diseases originating

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from rural settings suggest that biological agents present in animal breeding can pose a significant public health risk, and that risk might be higher in farmers and animal breeders. Existing data suggest that the risk of occupational zoonoses has been underestimated [8, 9]. It is therefore clear that in animal breeding settings, the health of animals, workers and consumers is 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, for instance, tetanus can be well controlled by immunization programmes [10]. *Clostridium tetani*, the causative agent of tetanus, is still a cause of disease and even fatalities among the Italian population. In 2009, the highest number of reported tetanus cases in the European Union countries was from Italy, with 58 confirmed cases [11].

Evidence suggests that the people at higher risk of zoonoses are those living in rural areas, both in developed and developing countries, and in particular those working with animals [12]. Since epidemiologic studies regarding occupational zoonotic risks are scarce in Italy, this explorative study was carried out in a rural area of the Lombardy Region with the aim of defining, through the determination of specific IgG antibodies, the serologic evidence of contact with selected zoonotic agents among groups of agricultural workers engaged in different working activities at the workplace.

Finally, in order to define the need for specifically targeted preventive interventions, and considering the fact that in Italy tetanus vaccination is compulsory for agricultural workers, occupational health physicians should check workers' tetanus immunization status during their periodic workers' health surveillance, it was decided to integrate the presented study with an investigation on tetanus immunity in the study groups.

MATERIALS AND METHOD

Since breeders are supposed to be at higher risk compared to non-breeders, the presented research aimed at characterizing the biohazards comparing 'animal breeders' (exposed) and 'non-breeders' (controls). Two cross-sectional studies were conducted in 41 small size agricultural enterprises provided with occupational health surveillance at the workplace in the Lombardy region. The region is almost at 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. The region of Lombardy is first in Italy for farming, with a considerable production of meat, milk and dairy products, with about 74,500 farms which occupy an agricultural area of 1.41 million hectares (ha) (Fig.1). On the other hand, Lombardy is the national leader in pig breeding in the country, amounting to 5.5 million tons per year, which equals 45% of the total Italian production. There are more than 15,000 cattle farms, while pig farms amount to around 4,000 [13].

The two studies were conducted from January 2010 – January 2011 on 2 separate groups consisting of 199 randomly selected workers from 41 farms in the region, as described below.

The first group (n=103) consisted of 47 cow breeders, 31 pig breeders, 11 fish breeders, and 14 non- breeders who were not involved in animal breeding activities. 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, the workers' sera was tested with 2 different kits: Assay 1- commercial enzyme immunoassay (EIA) kit (HEV Ab, DiaPro Diagnostic Bioprobes, Milan, Italy), and Assay 2- HEV IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise, China).

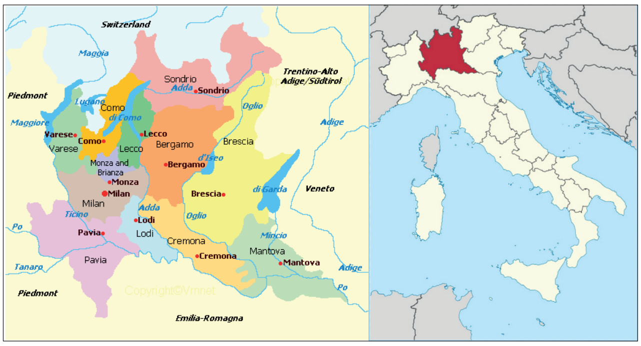

Figure 1. Lombardy region, northern Italy.

The second group (96 workers), included 28 pig breeders, 36 cattle breeders, and 32 workers who were not engaged in animal breeding (control group). All these subjects were tested for antibodies against *Salmonella spp., Brucella spp., Coxiella burnetii, Leptospira spp. and Borrelia burgdorferi* and for the levels of protection against tetanus (IgG). 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. 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 routinely used by our centre to collect data originating from individual health surveillance activities. Before the 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 analysis.

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 by the Chemiluminescence method (Diasorin, Italy), and the concentrations of antibodies against *Brucella spp* and *Salmonella spp* were measured with a Direct Agglutination method (Diesse, Italy). Concentrations of antibody to tetanus toxin (antiTT) in sera were measured by an enzyme-linked immunosorbent assay. Anti-TT levels equal or more than 0.11 IU/mL were considered protective.

Statistical analysis. Breeders and non-breeders were compared using the Mann-Whitney tset for continuous variables, while the chi-square test was used for categorical variables. In the first group of 103 workers the prevalence of anti-HEV antibodies in their sera 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 (control group), adjusted for age, gender, country of origin (EU vs. non-EU) and smoking (yes, no). Statistical analyses were carried out with the SPSS version 18 software (SPSS Inc., Chicago, IL, USA).

RESULTS

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

Table 1. Demographic and personal information of the first study groups (breeders/non breeders) n=103.

Workers/ Variables	Age (years) Median (Min-Max)	European Nation-ality (%)	Smokers (%)	Cigarettes/ day Median (Min-Max)	Alcohol con-sump-tion (%)	Alcohol-units/ day Median (Min-Max)
Breeders (n=89)	48 (27–71)	83.1	25.0	20 (5–40)	39.3	2 (1–6)
Non-breeders (n=14)	45 (30–75)	100	28.5	25 (20–30)	21.4	2 (1–3)
P value	0.76*	0.09**	0.35**	0.07*	0.9**	0.85*

* From Mann-Whitney test
** From chi-square test.

Hepatis E antibodies. 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 (1%). Since the subject was an Indian cattle breeder, it was assumed that it was 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, the results were completely different from those obtained with the first kit and the prevalence of antibody titres rose from 1 to 22.3%. Table 2 compares the results of two commercial anti HEV IgG assays on study subjects.

Table 2. Comparison of two anti-HEV IgG assays used in the study (n=103).

Workers	Assay I		Assay 2	
	+	%	+	%
Breeders	1/90	1	23/90	25.6
Non-breeders	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 19–70 (Median 42 years). Socio-demographic information related to the second group is shown in Table 3.

The odds ratios and 95% confidence intervals of seropositivity to antibodies against selected zoonotic agents in agricultural workers are shown in Table 4. Results of logistic regression analysis, adjusted for age, gender, country of origin and smoking habits revealed in breeder workers a tendency to have a higher prevalence of positivity for antibodies to *Leptospira spp.* and *Coxiella burnetii* than non-breeders, more evident in the multiple logistic regression analyses (ORs around 3). Little difference was found for *Borrelia burgdorferi* in the crude analysis, and a multiple regression analysis was not performed because of the small number of positives. None of the subjects showed antibodies against *Salmonella spp* and *Brucella spp.*

Table 3. Demographic and personal information of the second study groups (n=96).

Workers/ Variables	Age (years) Median (Min-Max)	European Nation-ality (%)	Smokers (%)	Cigarettes/ day Median (Min-Max)	Alcohol con-sump-tion (%)	Alcohol -units/ day Median (Min-Max)
Breeders (n=64)	44 (19–70)	65.6	21.9	17.5 (2–30)	41.0	2 (1–12)
Non-breeders (n=32)	34 (22–62)	87.5	34.4	10 (5–25)	66.0	1.5 (1–5)
P value	0.01*	0.02**	0.22**	0.63*	0.02**	0.21*

* From Mann-Whitney test
** From chi-square test.

Table 4. Prevalence of 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 (n=96).

Zoonotic agent	positive		OR Crude	95% CI	OR Adjusted*	95% CI
	No.	%				
Leptospira spp.						
Non-breeders	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 burnetii						
Non-breeders	10	31.2	1.00	(Reference)	1.00	(Reference)
Breeders	32	50.0	2.10	0.86,5.16	2.97	0.92,9.58
Swine-breeders	*14*	*50.0*	*2.10*	*0.73,6.04*	*2.91*	*0.81,10.45*
Cattle-breeders	*18*	*50.0*	*2.10*	*0.77,5.69*	*3.05*	*0.82,11.37*
Borrelia burgdorferi						
Non-breeders	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, gender, country of origin (EU vs. non-EU), smoking (yes, no)
NE – not estimated.

Immunization of workers against tetanus was investigated through the detection of anti-tetanus toxin IgG. Figure 2 represents immunity to tetanus in agricultural workers (first group, n=96). As the figure suggests, European workers might have better protection to tetanus compared to non-EU workers; in particular, tetanus immunity long-term protection (1–5 IU/ml) was present in 34 (48.6%) EU and 10 (38.5%) non-EU workers, but the difference did not reach the levels of statistical significance (P = 0.38, chi-square test).

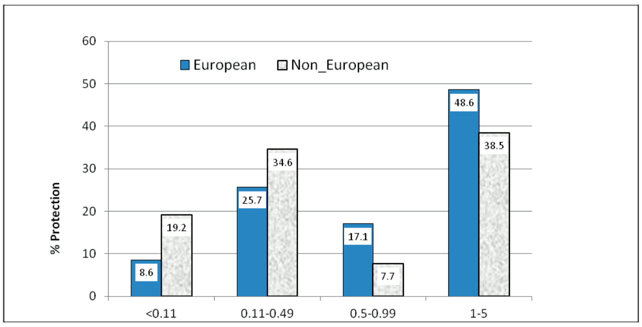

Figure 2. Comparing immunity to tetanus in agricultural workers by nationality (n=96)

DISCUSSION

The aim of the first study was to investigate the seroprevalence of antibodies against hepatitis E virus (HEV) in different animal breeders and the non-exposed group (non-breeders). Serological studies regarding HEV prevalence in Italy are scarce and little is known about occupational exposure in farms and animal breeder settings. Studies in Western Europe and United States indicate that the 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 seldom 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. Evidence suggests that people who consume contaminated pork products or are involved in the pigs breeding activities are potentially at risk of HEV infection [14].

Some studies suggest that HEV may be an asymptomatic zoonotic infection in industrialized countries [15, 16, 17]. Galiana et al. (2008) carried out a study in Spain 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 [18]. Their results indicated that swine breeders suffered a 5.4 times higher risk of having anti-HEV IgG compared to the control group (P = 0.03). In this light, HEV infection should be seriously considered as a possible occupational illness in these workers. As for Italy, the prevalence of anti-HEV IgG in the general population is estimated at around l – 5% [19, 20, 21, 22]. In the presented study, the prevalence of anti-HEV in agricultural workers determined with Assay 1 was 1%, a value approaching literature data, which also do not suggest significant differences for anti-HEV seroprevalence between swine farmers and the general population (2.9% and 3.3%, respectively), at least in the Latium region [23].

Enzyme immunoassays based on recombinant proteins of HEV have been used for most seroprevalence studies, and 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 immunoglobulin G (IgG) and IgM anti-HEV have not been well established in areas where hepatitis E is not endemic [24]. The presented study compared the sensitivity of 2 commercially available IgG anti-HEV kits. Results suggest that kit 2 is more sensitive than kit 1, but further investigation is needed in order to rule out any risk of misinterpretation of the results due to a high number of false positive as well as false negative results. It is worth mentioning that Mansuay et al., by using a validated sensitive assay (Assay 2), found hepatitis E virus (HEV) IgG in 52.5% of voluntary blood donors in southwestern France [25].

In the second pilot study, the authors of the presented study investigated other important zoonotic risks among agricultural workers, including Leptospirosis, one of the zoonotic infections widely seen, but especially in tropical regions. The primary transmission is by direct contact with water contaminated by the secretions of animals. Leptospirosis is endemic in many countries, perhaps even worldwide. It often has a seasonal distribution, increasing with increased rainfall or higher temperature [26]. Leptospirosis is of increasing importance as an occupational disease as intensive farming practices become more widely adopted. During 1999, people employed in agricultural industries in Australia accounted for 35.3% of notifications, while those working in livestock industries accounted for 22.9% of notifications [27].

In the presented study there was a relatively high prevalence of IgG to *Leptospira spp.* among workers (54.1%), which is higher than that reported in previous studies (5.6 – 40%) [28, 29]. Leptospirosis is still a public and occupational health problem in Italy: in the 3-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 the results implied that 18.2% of these cases were by direct contact with animals, specially swine [30]. 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 animal and 5.6% of human sera scored positive for the presence of antibodies to *Leptospira* [28]. Evidence suggest that until the 1960s, Leptospirosis was a moderately common occupational infection, especially in rice workers, due to the prevalence of wild rats [31]. Mono et al. (2009) carried out a survey in southern Italy to investigate the seroprevalence of zoonoses in 2 cohorts of farm workers and blood donors [32]. None of the subjects had antibodies against *Brucella* and *Salmonella*. Serologic studies on animals have shown different results for *Leptospira*, from 18% in wild boars in Germany [33], to 20 – 53% in rodents in France [34]. According to a WHO report, the 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 100,000 [35]. Overall, the presented study suggests that contact with *Leptospira spp.* is very common in agricultural workers, and that the risk is not related with breeding activities but only with a possible contact with surface water, possibly contaminated by rodents' urine.

In the current study, one of the zoonotic agents with a higher prevalence in the animal breeders, compared to non-breeders, was *Coxiella burnetii*. 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 the EU region, there was an outbreak of Q fever in the Netherlands in 2007, and by August 2009 more than 2,000 cases had been reported, with 11 fatalities [6]. Despite the widespread prevalence of infection with *Coxiella burnetii*, there have been few large population-based surveys that investigated the epidemiology of this infection [36]. Thomas et al. (1995) conducted a survey in the United Kingdom to investigate the 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. burnetii* and no association was found between seoprevalence and age. It was concluded that the risk of having antibodies to *C. 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 [37]. It is important to remember that an effctive vaccine against Q fever is available in Italy and throughout the whole of the European Union: the results of the presented study therefore 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. reported an outbreak of 58 cases during the summer and autumn of 1993 in the Veneto region in northeast Italy. The case control study showed a significant association with exposure to flocks of sheep [38]. In Germany, 2 outbreaks of Q fever were investigated: one occurred in 1992 in a Berlin research facility where sheep were kept, and the other in 1993 in a rural area in Hesse province. In both outbreaks, infected sheep were suspected to be the source of the outbreaks [39]. Santoro et al. (2004) reported an outbreak of Q fever in Como, northern Italy in 2003 [40], during which 133 cases of acute Q fever with clinical symptoms (high fever, dry cough, arythromyalgia, fatigue and chest abnormalities) with confirming serologic results, were reported to the prevention department of the local health unit (ASL). In this case, infected sheep were also suspected to be the source of the outbreaks.

The results of the presented study show that 42 workers out of 96 (43.7%), mainly breeders workers, had serum anti *Coxiella* IgG, thus suggesting that contact with animals is associated with increasing antibodies in workers' peripheral blood. One of the study limitations was that there was no opportunity to investigate the prevalence of zoonoses in sheep and goat breeders in the region.

Lyme diseease (LD) 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 [6, 41]. In Europe, there have been some seroprevalence studies on humans, where the highest incidences of LB are found in the Baltic States and Sweden in the north, and in Austria, the Czech Republic, Germany, Slovenia and central Europe [42]. Chiemlewska-Badora investigated anti-*Borrelia burgdorferi* antibodies in groups of forestry workers, farmers and blood donors in the Lublin region of southeastern Poland [43]. The results showed that the highest rate of seropositivity was among forestry workers and farmers (38.6% and 28.1%, respectively), compared to 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 [44]. The results of the presented study indicate that 9.4% of the workers studied had anti-*Borrelia* IgG in their serums, which is higher than the results obtained by Di Renzi et al. in forestry workers (3.4%), and below the values of Chiemlewska-Badora study results in the Lublin region of southeast Poland (13–33% in exposed groups) [45, 46]. The other point is that the laboratory results of the presented study imply less anti-*Borrelia* antibodies in cattle-breeders, compared to pig breeders and non-breeders (2.8%, 14.3% and 12.5%, respectively).

The presented study also investigated tetanus immunity in agricultural workers, considering the fact that tetanus remains an important public health problem in developing countries, but cases also occur, although rarely, in developed countries [47]. According to the European Centre for Disease Control and Prevention (ECDC) Report, in 2009 there were 128 tetanus cases, including 79 confirmed cases, reported by 27 countries (Italy had 58 confirmed cases). The overall notification rate was 0.02 per 100,000 population, and the highest rate was reported by Italy (0.1 per 100,000) [11]. Overall, tetanus has become a disease of the elderly, with 50% of cases of tetanus occurring in persons aged 65 and over [48]. The case-fatality rate also increases with age and reaches 50% for patients above the age of 60 [49]. The incidence of reported tetanus in Italy decreased from 0.5/100,000 in the 1970s to 0.2/100 000 in the 1990s. According to Sangali et al., about 40 deaths due to tetanus were reported in Lazio, Italy, during 1985–1994, and that retired persons accounted for 48% of all deaths [50]. The results of the presented study show that only 48.6% (IT/EU) and 38.5% (Non-EU) of the workers had long-term protection against tetanus; on the other hand, a decline in the level of the anti-tetanus antibody was seen with the increase in the age of workers, which is consistent with literature.

The other finding of the presented study was a tendency of migrant workers towards a lower coverage of tetanus immunity, compared to Italian/EU workers (81% and 91%, respectively), while 19.2% had no immunity, compared to EU workers (8.6%). Therefore, more attention must be paid to anti-tetanus vaccination and booster doses for agricultural workers.

In general, the results of the presented study through statistic elaborations, including univariate and multivariate regression analysis, suggest that animal breeders have a higher percentage of antibodies to *Coxiella spp.* and *Leptospira spp.,* compared to other workers not engaged in animal breeding activities.

CONCLUSIONS

The high percentage of anti-*Coxiella* and *Leptospira* antibodies found in agricultural workers occupationally exposed to zoonotic risks may suggest that these agents might be widespread in northern Italy. There was no anti-*Brucell*a and *Salmonella* IgG detected which might reflect the fact that this area is clear from these 2 important zoonotic diseases. The results suggest that two zoonoses, especially *Coxiella* and *Leptospira,* in northern Italy may have a work-related character and all agricultural workers (breeders and non-breeders), irrespective of their contact with animals, are counted as groups at risk because of the ubiquitous presence of these risk factors in the rural environment (e.g. *Leptospira* in surface water contaminated by rodent 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 facilities or poor surveillance of these diseases. More attention must be paid to tetanus immunity among workers, especially migrants, and subjects needing a booster dose of tetanus vaccine, as well as vaccination, should be identified.

Finally, the presented data draw attention to the need to confirm and extend seroprevalence studies in broader high risk populations in order to better define the risk and, if necessary, take appropriate measures to prevent zoonotic diseases.

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