Journal of Central European Agriculture, 2015, 16(4), p.357-369 DOI: 10.5513/JCEA01/16.4.1632

Importance of microbiological research of bioaerosols during horse breeding

Znaczenie badań mikrobiologicznych bioaerozoli podczas hodowli koni

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Abstract

The aim of this study was to estimate microbiological quality of bioaerosols sampled from indoor and outdoor air of stables. Two types of closed stables (type 1 and 2) and one opened were tested during winter, spring and summer periods. Summary the highest number of bacteria was in closed stable type 2 (above 10^5 CFU×m^{-3}), then opened (up to 10^5 CFU×m^{-3}) and stable type 1 (near low 10^4 CFU×m^{-3}). The number of fungi did not exceed 10^4 CFU×m^{-3} and was the greatest in stable type 1. Pool of mesophilic bacteria isolated from bioaerosols of both closed stables represented almost the same genetic profile, as well as between psychrophilic bacteria isolated from opened stable and background.

Keywords: air sampling, bacteria, bioaerosol, fungi, horse breeding, stable

Streszczenie

Celem przeprowadzonych badań była ocena jakości mikrobiologicznej bioaerozoli pobranych wewnątrz i na zewnątrz pomieszczeń stajennych. Oceniano jakość powietrza w dwóch typach stajni zamkniętej (boksowej i angielskiej) oraz otwartej, w trakcie trwania sezonu zimowego, wiosennego i letniego. Najwyższą ogólną liczebność bakterii otrzymano w stajni typu angielskiego (powyżej 10^5 jtk×m^{-3}), następnie otwartego (do 10^5 jtk×m^{-3}) i typu boksowego (blisko dolnej granicy 10^4 jtk×m^{-3}). Liczba grzybów mikroskopowych nie przekraczała wartości 10^4 jtk×m^{-3} i była największa w stajni typu boksowego. Pula bakterii mezofilowych wyizolowanych ze stajni zamkniętych typu boksowego i angielskiego ujawniła niemal identyczny profil genetyczny, podobnie jak w przypadku psychrofilowych bakterii izolowanych ze stajni otwartej i powietrza atmosferycznego w otoczeniu obiektów stajennych.

Słowa kluczowe: badanie powietrza, bakterie, bioaerozole, grzyby mikroskopowe, hodowla koni, stajnia

Streszczenie szczegółowe

Hodowla koni wiąże sie ze sportem, rekreacją, pracą oraz z produkcją żywności. Spożywanie mięsa końskiego należy do narodowych tradycji kulinarnych niektórych krajów ze względu na wysoką zawartość mikroelementów i witamin. Koń jest zwierzęciem bardzo czułym na zanieczyszczenie powietrza i jednocześnie kłopotliwym w trakcie leczenia ewentualnych powikłań po infekcjach bakteryjnych i grzybiczych. Stosunkowo niewiele miejsca w literaturze poświęca się problematyce ładunku zanieczyszczeń mikrobiologicznych w stajniach. Dlatego celem pracy było określenie stopnia zanieczyszczenia powietrza (bioaerozoli) wewnątrz pomieszczeń stajennych i powietrza atmosferycznego wokół obiektów. Ocenie poddano bioaerozole w dwóch typach stajni zamkniętej (boksowej i angielskiej) oraz otwartej, w trakcie trwania sezonu zimowego, wiosennego i letniego. Powietrze do badań pobierano metodą zderzeniową, dwoma próbnikami powietrza oraz sedymentacyjną na płytki Petriego z odpowiednimi pożywkami mikrobiologicznymi dla bakterii i grzybów mikroskopowych. Próbki bioaerozolu pobierano z wysokości 1.2 metra umieszczając próbniki i płytki na powierzchni płaskiej i odkażonej o wielkości 1 metr x 1 metr. Jednocześnie mierzono temperaturę, wilgotność, siłę i kierunek wiatru. Mikroorganizmy zebrane na płytkach hodowano w warunkach optymalnej temperatury, czasu i wilgotności. Zakres temperatur w jakich prowadzono badania w czasie trzech kwartałów wyniósł od 2°C w zimie do 44°C w lecie. Badania na zewnątrz pomieszczeń stajennych wykonywano przeważnie podczas słonecznej pogody bez opadów atmosferycznych a zakres wilgotności wahał się od 25% do 98%. Wewnątrz pomieszczeń wilgotność była względnie stabilna i wynosiła około 75% z maksimum w stajni typu boksowego (98%). Najliczniej w bioaerozolach występowały bakterie psychrofilne i *Pseudomonas* sp. Maksimum występowania wszystkich badanych mikroorganizmów przypadł na okres lata. Różnice w ogólnej liczebności mikroorganizmów pomieszczeń stajennych w okresie wiosennym i zimowym okazały się nieistotne statystycznie. Najwyższą ogólną liczebność bakterii otrzymano w stajni typu angielskiego (powyżej 10^5 jtk×m^{-3}), następnie otwartego (do 10^5 jtk×m^{-3}) i typu boksowego (blisko dolnej granicy 10^4 jtk×m^{-3}). Liczba grzybów mikroskopowych nie przekraczała wartości 10^4 jtk×m^{-3} i była największa w stajni typu boksowego. Analiza korelacji na poziomie istotności p<0,95ujawniła wzrost liczby bakterii mezofilowych jednocześnie z psychrofilowymi I grzybami. Szczególnie wysoką dodatnią korelację ujawniono dla bakterii hodowanych na agarze z krwią i gronkowców. Uwzględniając cały okres badań gradacja ładunku zanieczyszczeń bioaerozolu była skierowana od najniższego dla hali ujeżdżeniowej (4.2 × 10^3 CFU×m^{-3}) do dziesięciokrotnie wyższego dla stajni angielskiej (4.3 × 10^4 CFU×m^{-3}). Najbardziej zanieczyszczona okazała się stajnia boksowa w sezonie letnim (1.2×10^5 CFU×m^{-3}). Grzyby mikroskopowe dominowały liczebnie również w stajni boksowej. Po wykonaniu analiz mikrobiologicznych izolowano DNA z pojedynczych kolonii (jtk), oczyszczano materiał genetyczny i amplifikowano metodą RAPD-PCR z rozdziałem elektroforetycznym. Uzyskane wzory prążkowe poddawano analizowano i określano podobieństwo genetyczne pomiędzy izolatami. Pula bakterii mezofilowych wyizolowanych ze stajni zamkniętych typu boksowego i angielskiego ujawniła niemal identyczny profil genetyczny, podobnie jak w przypadku psychrofilowych bakterii izolowanych ze stajni otwartej i powietrza atmosferycznego w otoczeniu obiektów stajennych.

Introduction

The horse breeding complies aspects of sport, recreation, work as draft horses. In some country horse meat is important part of culinary tradition because it has height content of microelements and vitamins. All these aspects require proper management especially good balanced nutrition, selection for desirable genetic traits, reproduction, the highest welfare. Maintenance of high welfare supports all aspects of horse breeding. In stable style of breeding one of the most important aspect is good quality of air, especially in winter and spring when horses spend the most of their time in stable. Stabled horse, as naturally adapted to live in the open area, it is exposed to high concentration of pathogenic bacteria and fungi then in the environment. House keeping especially for horse kept in stalls, fed hay and bedded on straw, plays great impact on lungs and if it is not properly realised may cause permanent damage e.g. chronic obstructive pulmonary disease (C.O.P.D). Air with pathogenic microorganisms from litter and hay is inhaling by horse kept in closed stable being the reason of recurrent airway obstruction (R.A.O). Moreover health problem is bidirectional between horse and horse enthusiasts or/and caretakers. Man could be affected by harmful bioaerosols from stable then shows bronchial obstruction. Simultaneously, the more pathogenic bacteria and fungi, the more endo- and egzotoxins exist in the air. Many small particles defining the respirable dust concentration (R.D.C) has diameter about up to 5 micrometers as many of viruses, bacteria and spores of fungi. Bacteria are able to enter the peripheral airways and actively cause pulmonary inflammation

The most harmful bacteria for horse‘s and man’s respiratory tract and other internal organs are among others enterobacteria, vibrio, coccoid bacteria and corynebacteria. Anyway and with no discussions, problem of good quality of air into stables is crucial to realise and that is why we undertook this problem in microbiological point of view.

Materials and Methods

Two types of closed stables and one opened were tested: type 1 called in polish as “box type”, type 2 called in polish as “english type” and opened stable. In type 1 closed boxes are each about 10 square meters of area. In building were seven horses. In type two opened boxes are each about 11 square meters of area. In such building were 10 horses. In opened building were 3 horses on 40 square meters of area.

Air was sampled inside (indoor) and outside (outdoor) of stables. Minimum three places of sampling area (N) were established on 120 cm above the ground. Outdoor area of stables, named as background, was situated 20 to 50 meters away of stabling horses. Sedimentation method was done on standard Petri plates (90 mm of diameter) for 10 and 30 minutes of exposure. Impact method we realised by MAS-100 Eco (Merck, KgaA, Germany) and Biocollector BC100 (AWEL International, France). Samples of the air were collected when routine morning activities were begin carried out, over a period of 4 hours to obtain a peak exposure values of CFU. The air was sampled during winter (February), spring (April/May), and summer (August). Thermal conditions (°C) and humidity level (%) inside and outside of stables were monitored.

Bacteria were cultured on Tripticase Soy Agar (TSA; Merck, 1.05458) at 37°C by 24 h, agar with 5% of defibrynated sheep blood at 37°C by 24 h, Mannitol Salt Agar

(according Chapman) at 35 °C by 48 h, Pseudomonas CFC selective agar (Merck, 1.07620 suppl. 1.07627) at 25 °C by 48 h, XLD LAB-Agar (Biocorp, PP1330) at 37°C by 24 h, Salmonella-Shigella Lab-Agar (Biocorp, PP1250) at 37°C by 24 h, and recorded as colony forming units (CFU). Additional catalase-oxidase, Hugh-Leifson tests and RAPD-PCR (OPB-10) reactions were done for strain confirmation and preliminary solving of epidemiological aspects (Williams et al. 1990). Simultaneously, number and diversity of fungi were tested on Rose-Bengal Chloramphenicol Agar (Merck, 1.00467) and Sabouraud (Merck, 1.05438) as supporting medium both at 25 °C by 7 days. Colonies of fungi were removed, fixed and preserved for microscopic analysis of taxonomic membership.

Data were verified by one factor analysis of variance with significance of differences between the mean values of CFU by Duncan's test. Epidemiological aspects were dissolved by neighbour joining method analysis. All done by using of Statistica 5.0 and PopGene32 software.

Results

Indoor temperature and humidity during study were between 2 to 4°C and 76 to 98% in winter; 15 to 19°C and 58% in spring, 25 to 44°C and 25 to 74% in summer, respectively. Upper level of temperature with lower humidity were observed outside of buildings and marked as background. Maximum wind speed on the background never exceed 4 $m \times s^{-1}$. The most abundant group of microorganisms isolated from bioaerosols of stable were psychrophilic bacteria and bacteria *Pseudomonas* sp. (Table 1). The peak of occurrence for all microorganisms in bioaerosols in stables appeared in a summer. Predominately, there were statistical differences between number of bacteria collected in winter and summer and in spring and summer. Differences of values between winter and spring were not statistically significant.

Table 1. Average values of bacteria isolated from particular seasons ($CFU \times m^{-3}$)

Tabela 1. Średnia liczba bakterii izolowanych w poszczególnych porach roku ($jtk \times m^{-3}$)

Season	Meso	Psychr	Bl-a	α/β haemo	Staph. sp	Staph. a/s	Pseudo	Fungi
Winter	160^{A}	124^{A}	Nd	nd	157^{A}	157^{A}	179^{A}	172^{A}
Spring	536^{A}	1373^{A}	465^{A}	258^{A}	301^{A}	264^{Aa}	716^{A}	207^{A}
Summer	14709^{B}	51448^{B}	38177^{B}	32636^{B}	16008^{B}	14424^{Ab}	47928^{B}	9029^{B}

A – at e level of p<0,01; a – at a level p<0,05; Meso – mesophilic; Psychr – psychrophilic; Bl-a – agar with blood; α/β haemo – haemolytic; Staph. sp – *Staphylococcus* sp.; Staph. a/s – *Staphylococcus aureus* and *Staphylococcus saprophyticus*; Pseudo – *Pseudomonas* sp; Fungi – yeast and mould

Almost in all cases number of haemolytic bacteria was equal with general number on blood agar. The same situation it was revealed in relation to mannitol positive staphylococcus versus general number of bacteria on mannitol salt agar. In case of

fungi mostly *Alternaria* sp., *Fusarium* sp., *Scopulariopsis* sp., *Penicillium* sp., *Aspergillus* sp. were found on microscopic slides but in relation to bacteria moulds and yeast were in minority. Only in winter their level was equal to bacteria.

Analysis of correlations at $p<0.95$ obtained positive reaction of increasing number for mesophiles together with psychrophilic bacteria and fungi. Positive correlation was calculated for psychrophilic bacteria and all microorganisms in bioaerosols. The highest positive correlations occurred for bacteria incubated on blood agar and staphylococcus. The number of CFU for fungi in air of stables elevated slightly together with increasing of *Pseudomonas* sp.

Taking into consideration of whole period of research (three seasons) the gradation of microbiological load as pollution for air was from riding hall (4.2×10^3 CFU×m^{-3}) then statistically the same for stable type 1 and triplicate higher for opened stable and above 10 times higher for stable type 2 (4.3×10^4 CFU×m^{-3}). The most pollutant was stable type 1 in summer (1.2×10^5 CFU×m^{-3}) and the clearest air was into stable type 1 in winter (8×10^1 CFU×m^{-3})(Figure 1). Fungi dominated in stable type 1 in summer (1.7×10^4 CFU×m^{-3}). In opened type of stock building fungi were in minority in spring season. The number of bacteria in bioaerosols with particular data for type of stable and kind of bacteria are compared into tables 2, 3 and 4.

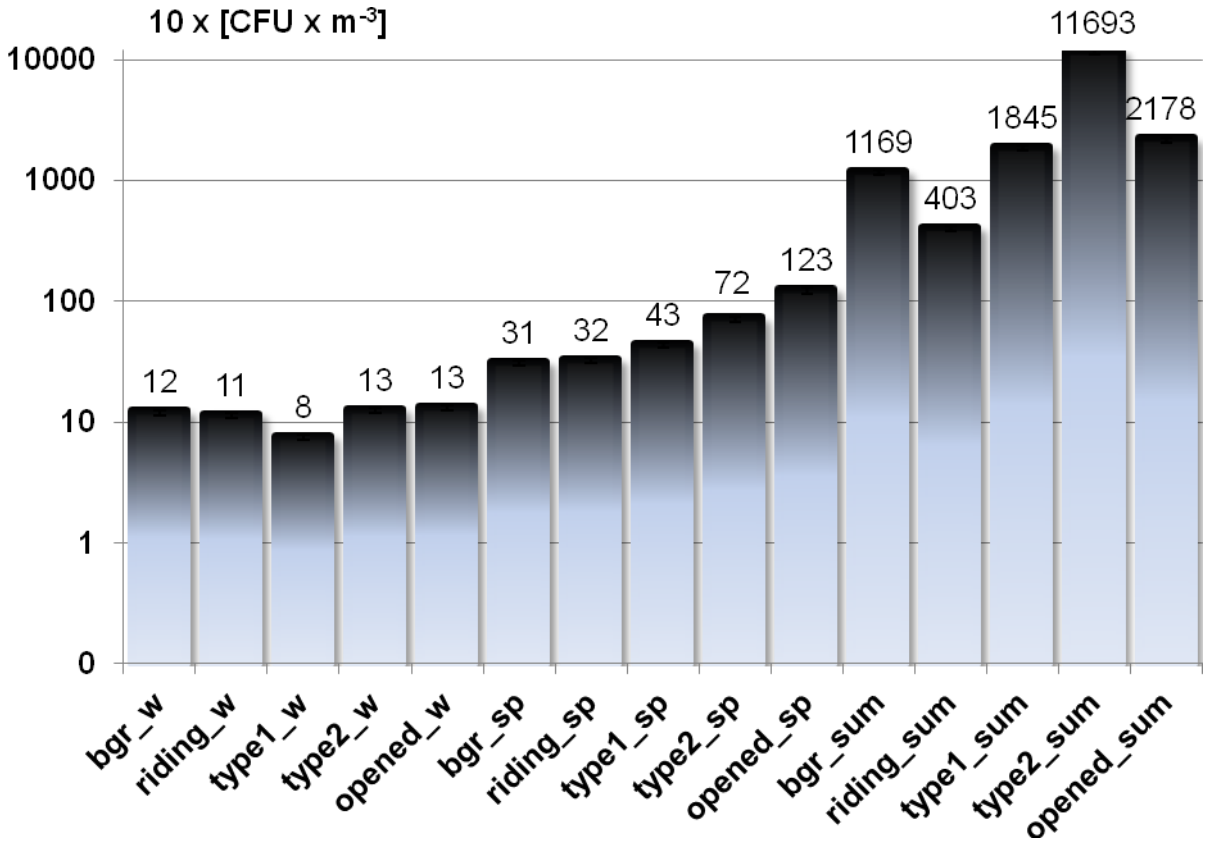


Figure 1. Number of bacteria in the air of stables

Ilustracja 1. Liczba bakterii w powietrzu stajni

The number of bacteria incubated on blood agar and TSA at 37ºC were similar for spring and statistically different in summer (Table 2). Percent of alpha and beta haemolytic bacteria on blood agar was from 5 stable type 1 in summer up to near 100 in opened during spring period summer. Summary the highest percent of haemolytic bacteria was in opened stable. Predominately CFU on blood agar represented β-haemolysis, then α-type.

Table 2. Number of bacteria (x±SD) incubated on TSA and blood agar compared to the amount of alpha-beta haemolytic bacteria isolated from stables during particular seasons (CFU×m^{-3})

Tabela 2. Liczba bakterii (x±SD) izolowanych na TSA i agarze z krwią w porównaniu z liczbą bakterii alfa-beta hemolizującymi izolowanych ze stajni w poszczególnych porach roku (jtk×m^{-3})

Season	Stable	Mesophilic	Psychrophilic	Blood agar	α/β haemolytic
Winter	Background N=4	240±3	165±12	nd	nd
	Riding hall N=3	102±11	103±10	nd	nd
	Stable type 1 N=3	108±4	108±2	nd	nd
	Stable type 2 N=3	113±5	114±2	nd	nd
	Opened N=3	262±0	118±10	nd	nd
Spring	Background N=6	306±47	357±23	230±28	63±7
	Riding hall N=3	233±11	1381±10	149±0	44±0
	Stable type 1 N=6	471±40	1152±76	350±20	61±3
	Stable type 2 N=6	563±140	2231±155	508±83	195±11
	Opened N=3	1381±10	2127±10	1393±10	1381±13
Summer	Background N=8	11794±875	42837±193	8280±71	5360±85
	Riding hall N=6	2219±27	14637±701	2202±240	443±55
	Stable type 1 N=8	10288±732	30555±1222	20471±264	1118±148
	Stable type 2 N=6	19889±206	110543±1272	174107±2346	170161±2401
	Opened N=8	32269±619	67279±1679	15723±791	13398±1120

N – number of samples; SD – standard deviation; nd – no data

Selected CFU tested on API 20-NE, HF test and microscopic staining were identified as *Staphylococcus* sp, probably (90%) *Staphylococcus aureus* and *Staphylococcus saprophyticus.* In samples of air incubated on mannitol salt agar it was confirmed presence *Staphylococcus* sp. including dominating *S. aureus* and *S. saprophyticus* at equal level (Table 2). In winter level of CFU for mannitol positive *Staphylococcus* sp. was almost equal for all stables (99%) in opened stable in winter (100%).

Table 3. Average number (x±SD) of bacteria on blood agar, alpha-beta haemolytic and mannitol salt agar isolated from stables during particular seasons (CFU×m^{-3})

Tabela 3. Średnia liczba (x±SD) bakterii na agarze z krwią, alfa-beta hemolizujących i mannitolo- zmiennych izolowanych ze stajni w poszczególnych porach roku (jtk×m^{-3})

Season	Stable	Blood agar	α/β haemolytic	Staph. sp	Staph. a/s
Winter	Background N=4	Nd	nd	123±17	123±17
	Riding hall N=3	Nd	nd	172±10	172±10
	Stable type 1 N=3	Nd	nd	106±4	106±4
	Stable type 2 N=3	Nd	nd	271±22	271±22
	Opened N=3	Nd	nd	88±10	88±10
Spring	Background N=6	230±28	63±7	476±66	357±50
	Riding hall N=3	149±0	44±0	4±0	2±0
	Stable type 1 N=6	350±20	61±3	26±15	16±9
	Stable type 2 N=6	508±83	195±11	256±24	238±27
	Opened N=3	1393±10	1381±13	889±10	889±10
Summer	Background N=8	8280±71	5360±85	987±10	872±89
	Riding hall N=6	2202±240	443±55	1844±18	215±12
	Stable type 1 N=8	20471±264	1118±148	12814±170	12387±166
	Stable type 2 N=6	174107±2346	170161±2401	71220±931	71220±931
	Opened N=8	15723±791	13398±1120	8195±731	979±41

N – number of samples; SD – standard deviation; nd – no data; Staph. sp – *Staphylococcus* sp.; Staph. a/s – *Staphylococcus aureus* and *Staphylococcus saprophyticus*;

In summer mannitol positive bacteria were about 24% of general number in opened stable up to near 100% in stable type 2 (Table 3).

Percent of mannitol positive bacteria in pool of haemolytic were in range between 13 in opened stable during summer up to 64 in spring. In some cases, especially for stable type 1 and 2 in summer, values of CFU revealed on mannitol salt agar were 100 times higher than haemolytic bacteria on blood agar (Table 3). Numerous *Pseudomonas* sp. were in air of stable type 2 in summer (Table 4). Relatively small number of this microorganisms was revealed in opened stable and riding hall in winter season.

Table 4. Average number (x±SD) of Pseudomonas and microscopic fungi on the background of mesophilic and psychrophilic bacteria isolated from stables during particular seasons (CFU×m^{-3})

Tabela 4. Średnia liczba (x±SD) Pseudomonas i grzybów na tle bakterii mezofilowych i psychrofilowych izolowanych ze stajni w poszczególnych porach roku (jtk×m^{-3})

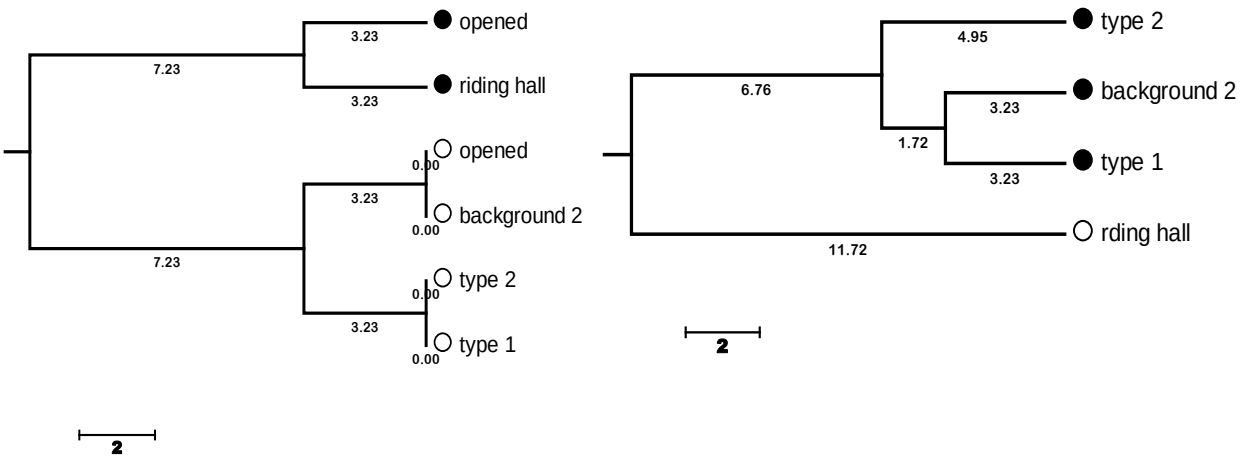
Season	Stable	Mesophilic	Psychrophilic	Pseudo. sp.	yeast/mould
Winter	Background N=4	240±3	165±12	197±27	367±33
	Riding hall N=3	102±11	103±10	250±10	101±10
	Stable type 1 N=3	108±4	108±2	105±2	106±2
	Stable type 2 N=3	113±5	114±2	111±2	111±2
	Opened N=3	262±0	118±10	365±10	110±10
Spring	Background N=6	306±47	357±23	383±48	262±13
	Riding hall N=3	233±11	1381±10	456±10	10±0
	Stable type 1 N=6	471±40	1152±76	959±42	388±38
	Stable type 2 N=6	563±140	2231±155	1026±20	159±66
	Opened N=3	1381±10	2127±10	534±10	28±0
Summer	Background N=8	11794±875	42837±193	11687±700	3233±49
	Riding hall N=6	2219±27	14637±701	6677±927	1234±164
	Stable type 1 N=8	10288±732	30555±1222	41544±506	17281±2402
	Stable type 2 N=6	19889±206	110543±1272	201379±2667	13791±1773
	Opened N=8	32269±619	67279±1679	14596±902	15406±667

N – number of samples; SD – standard deviation; Pseudo. sp – *Pseudomonas* sp.

Yeast and mould were the most abundant in summer season of the year, especially in the air of stable type 1, then opened and stable type 2 (Table 4). In relation to mean value of bacteria incubated on TSA (37°C and 22°C together) fungi stated from 2% in opened stable during spring up to near 100% in riding hall, stable type 1 and 2 during winter.

Considering background as datum point (100%) in winter mesophilic bacteria were below except in opened stable (110%), psychrophilic with fungi were below point, *Staphylococcus* sp were below except riding hall (141%) and stable type 2 (222%), and finally for *Pseudomonas* sp. below point were only stable type 1 and 2. In spring below datum point was practically riding hall except psychrophiles (387%) and *Pseudomonas* sp (119%). The same picture of spring was mapped as summer results but in lower values and with one exception for riding hall in relation to *Staphylococcus* sp. (187%). In relation to bacterial level in air of background maximum peaks for stables were in summer for stable type 1 and 2, especially for blood agar bacteria, haemolytic and *Staphylococcus* sp.

Cluster analysis of whole pool isolated psychrophiles in the air, during winter revealed closest genetic relationships between stable type 1 and 2 stables (I_N = 1). High genetic similarity it was obtained for opened stable and background (I_N = 1). Analysis of whole pool of mesophiles revealed the highest genetic identity for bacteria isolated from opened stable and riding hall (I_N=0,94) as well as stable type 1 and 2 and background (Figure 2).



dark circles show mesophiles / light circles show psychrophiles

Figure 2. Dendrograms based on Nei's (1972) genetic distance for two clusters (UPGMA method)

Ilustracja 2. Dendrogramy typu UPGMA w oparciu o odległość genetyczną Nei'a (1972) dla dwóch klasterów

Discussion

The most harmful bacteria for horse‘s and man’s respiratory tract and other internal organs are among others enterobacteria, vibrio, coccoid bacteria and corynebacteria (Bergstrom, et al., 2012). All possess small size and can migrate through lower respiratory tract cause inflammation. Because that fact the most important factor to control in stable is quality of the air that horse is breathing. Dust in stock buildings contains fungi, spores, bacteria that migrate from outside natural environment, feed, bedding materials and join with droplets of water making dangerous bioaerosols. Some of them such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* are general agent of asymptomatic chronic obstructive pulmonary disease of the man’s lower airways (de Serres, et al., 2009; Menso, et al., 1995). In relation to horse another medical term is used – R.A.O (Walinder, et al., 2011). Among fungi common are *Penicillium* sp., *Aspergillus* (*A. fumigatus*, *A. niger*, *A. flavus*, *A.terreus*). Occasionally, other fungal taxa such as *Alternaria* sp., *Cladosporium* sp., *Fusarium* sp., *Beauveria* sp., *Drechslera* are recovered (Nardoni, et al., 2005).

Summary the highest number of bacteria in stables during summer is not a surprise cause of very high temperature outside of stock buildings with simultaneous lower temperature and highest humidity inside them. Solar waves, including ultra violet, damage bacteria decreasing their abundance in the air. Moreover there are a lot of flying insects inside buildings contacting with the surface of mediums on opened Petri plates in sedimentation method of air sampling. These all made the results overrated about one order of magnitude. Highest number of psychrophiles during summer is caused their biological characteristics such as relatively low optimal growth temperature, wide range of tolerated temperatures, against solar waves protective pigments of cell wall, relatively high resistance on drying. All types (groups) of bacteria especially *Staphylococcus* sp and predominately identified *Staphylococcus aureus* / *saprophyticus* and *Pseudomonas* sp. were very often in stable type 1, 2 and riding hall during summer and their level exceed two orders of magnitude in relation to background. Such results suggest possibility for loss of welfare balance and eventually presence of skin, ear, nose and mucous membrane pathological dysfunctions for man and horse (Schmid-Hempel and Frank, 2007, Weese, et al., 2005). In winter and spring periods numbers of bacteria were all below of infection doses. However, for riding hall and stable type 2 most pathogenic bacteria were two times frequent then in background. Reason of this was low temperature outside of buildings (from minus 5°C up to minus 10°C), lack of ventilation by air-hole blinded. Sealed up ventilation holes or specific construction of stable type 2, not provided any ventilation systems when it closed, help increasing number of bacteria in winter for stable type 2 and bacteria together with fungi in spring season. Higher temperatures outside of building in spring promote faster division of bacterial cells. Frequent leading out of horses promotes migration of dynamically dividing bacteria that in general are in invasion stadium through environment. Inside of stables tends good environment for further development of bacterial number. That is why in spring bacteria were abundant in almost all types of stables but in particular into opened stable, then stable type 2 and 1. This situation was provided by free playground, contact with domestic fowl and their excrements without limitations, huge number of spores and psychrophiles on fresh grass. All type of microorganisms were brought back from paddock into stables. In relation to stable type 1 and 2, horses lead out preferable only for riding school lessons, exercised on sand and pool of brought back

microorganisms were not so rich as in opened type. Moreover in spring stables are ventilated and air holes are opened. Based on literature our results are in consensus with obtained by Witkowska, et al. (2012) for number of fungi during four seasons of the year. Similar conception we undertook and convergent results we obtained as Nardoni, et al. (2005) and Walinder, et al. (2011).

There are a lot of methods for air sampling with their advantages and limitations (Walinder, et al., 2011). In our research preliminary comparison of two methods we conducted: sedimentation and impact by two samplers. Our observation confirmed by results, revealed higher number of CFU on Petri plates by sedimentation method than impact. Protected plate against insects, small holes of air entrance onto plate in samplers effectively limited contact allochtonic bacteria originated from insects' bodies. Difference in CFU were on level of about 25-50%. There were not additional differences in results between Mass-100 Eco and Biocollector BC100 except technical possibility to aspirate smaller amount of air (max 10 m^3) in second one. In some cases during summer reading of CFU on plates was difficult by the reason of overgrowth.

Molecular marker such as random amplified polymorphic DNA (RAPD) with OPA-10 primer revealed its strong nature in epidemiological aspect. Grouping after cluster analysis joined similar means as closely related for two clusters. In one cluster divided by two subclusters mesophilic bacteria revealed high fidelity of genetic patterns between bacteria isolated from opened stable and riding hall in on side and mesophilic bacteria isolated from opened stable, background as one and stable type 2 with stable type 1 in second side. Pool of bacteria isolated from opened stable will normally genetically very similar to field pool from natural environment in case of healthy horses. In case of health disorders these pools will be the more different the more unhealthy horses be. Little genetic distance between pools of mesophiles isolated from bioaerosols into stables type 1 and 2 with high distance to the background suggests presence of specific bacteria inside of stock buildings. Picture of genetic relations in second cluster obtained high fidelity of patterns we may explain as local diversification of taxa caused by rare leading out of horses, closed ventilation system, intensive use of horses for training.

Conclusions

Although in Polish legislation exist the lack of standards for air quality into stables and another stock building the problem of keeping best quality of air is a key. In some reason good quality of bioaerosols is fundamental for horse health especially in case of valuable races. In other hands in all cases best welfare is basic for good economic conditions for enthusiast, breeders, veterinary sector, sport management, school trainers. Harmful bacteria and fungi together with danger chemical compounds are all not neutral for horse's and man's respiratory tract. Some microbiological and molecular tools presented in this paper help for checking, tracking, monitoring and fast responding on biological and pathological factors influencing on wide understanding horse breeding. Reality is often different than theory in many aspects of horse breeding. Monitoring of microbiological pollution of the air in stables helps to control cleaning process in stables when horses should be lead out but they are not especially in recreation stables (many horses), sport stables (risk of injury), special stables (private horses with special permissions). Technically, horses remain in stables during maintenance activity, inhale dust, bioaerosols, bacteria, fungi, spores.

If we add to this the lack of ventilation system or closing stables during winter, the problem of health disorder will appear for both horse and man.

Acknowledges

Paper in part financed by University of Warmia and Mazury budget for students in research associations. Special thanks for professional support and substantial assistance in subject of horse breeding to PhD Magdalena Łuczyńska. For assistance during all field management, including air sampling, bacterial cultivation, result keeping and self presenting of results on several conferences special greetings to Magdalena Sosińska, v-ce president of Students' Research Association of Molecular Microbiologists „COCCUS".

References

Bergstrom, K., Aspan, A., Landen, A., Johnston, C., Gronlund-Andersson, U., (2012) The first nosocomial outbreak of methicillin-resistant *Staphylococcus aureus* in horses in Sweden. Acta Veterinaria Scandinavica, 54, 11. DOI:10.1186/1751-0147-54-11

De Serres, G., Lampron, N., La Forge, J., Rouleau, I., Bourbeau, J., Weiss, K., Barret, B., Boivin, G., (2009) Importance of viral and bacterial infections in chronic obstructive pulmonary disease exacerbations. Journal of Clinical Virology, 46, 129 – 133. DOI:10.1016/j.jcv.2009.07.010

Menso, E., Ruiz, J., Rosell, A., Manterola, J., Fiz, J., Morera, J., Ausina, V., (1995) Bacterial infection in chronic obstructive pulmonary disease. A study of stable and exacerbated outpatients using the protected specimen brush. American Journal of Respiratory and Critical Care Medicine, 152, 1316-1320. DOI: 10.1164/ajrccm.152.4.7551388

Nardoni, S., Mancianti, F., Sgorbini, M., Taccini, F., Corazza, M., (2005) Identification and seasonal distribution of airborne fungi in three horse stables in Italy. Mycopathologia, 160, 29 – 34. DOI:10.1007/s11046-005-2669-3

Schmid-Hempel, P., Frank, S.A., (2007) Pathogenesis, virulence, and infective dose. PLoS Pathogens, 3(10). DOI: 10.1371/journal.ppat.0030147

Walinder, R., Riihimaki, M., Bohlin, S., Hogstedt, C., Nordquist, T., Raine, A., Pringle, J., Elfman, L., (2011) Installation of mechanical ventilation in a horse stable: effects on air quality and human and equine airways. Environmental Health and Preventive Medicine, 16, 264 – 272. DOI: 10.1007/s12199-010-0195-5

Weese, J.S., Rousseau, J., Traub-Dargatz, J.L., Willey, B.M., McGeer, A.J., Low, D.E., (2005) Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses. Journal of the American Veterinary Medical Association, 226, 580-583. DOI: 10.2460/javma.2005.226.580

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18, 6531-653. DOI: 10.1093/nar/18.22.6531

Witkowska, D., Kwiatkowska-Stenzel, A., Jóźwiak, A., Chorąży, Ł., Wójcik, A., (2012) Microbiological contamination of air inside and around stables during different seasons of the year polish journal of environmental studies, 21, 1061 – 1066.